



UNIVERSIDADE FEDERAL DO PARANÁ

SANDRA LESLY SIRET MARTÍNEZ

TAXONOMÍA E DIVERSIDADE DOS HIDROIDES (CNIDARIA, HYDROZOOA,  
“ANTHOATHECATA”) DE CUBA.

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“ANTHOATHECATA”) DE CUBA.

Dissertação apresentada ao Programa de Pós-Graduação em Zoologia, Departamento de Zoologia, Universidade Federal do Paraná, como requisito parcial à obtenção do título de Mestre em Zoologia.

Orientadora: Profa. Dra. Maria Angélica Haddad  
Coorientadora: Dra. Susel Castellanos Iglesias

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## RESUMO GERAL

Hidroide é o nome comum da fase polipoide das espécies da Classe Hydrozoa, Filo Cnidaria. Hydrozoa e outras classes do Subfilo Medusozoa incluem, no ciclo de vida, uma alternância de gerações entre a fase polipoide, bentônica e assexuada, e a fase medusoide, pelágica e sexuada. Este grupo de cnidários é um dos mais diversos do ambiente bentônico consolidado, com mais de 3.200 espécies já descritas. Os objetivos deste trabalho foram realizar novos levantamentos de hidroides da Subclasse Anthoathecata (atecados), atualizar a lista de espécies desses organismos em Cuba e avaliar sua diversidade e distribuição em dois ecossistemas marinhos da plataforma rasa, recifes de corais e gramas marinhas. A diversidade dos atecados foi ainda relacionada com indicadores de contaminação orgânica nos recifes de coral e com indicadores de qualidade da água em gramas marinhas. As coletas foram feitas em Março/2013 e Maio/2011, respectivamente, em recifes de corais de norte Havana e em pradarias de gramas marinhas em Caibarien, Villa Clara. Em mergulho autônomo, as técnicas de coleta foram censos visuais direcionados à raspagem dos hidroides, ao longo de transecções, e censos indiretos de raspagem total dos organismos de substratos delimitados por quadrados, onde toda a fauna e flora foram coletadas para analisar a presença de hidroides epibiontes. As amostras foram anestesiadas com mentol e fixadas em formalina 4%. Os hidroides foram separados dos substratos sob estereomicroscópio e para a identificação das espécies foram preparadas lâminas e fotografias em microscópios. As identificações foram embasadas principalmente nos estudos taxonômicos de Cuba, Caribe e região tropical do Atlântico Ocidental. Para confirmar as novas ocorrências, ampla literatura foi consultada. Da revisão bibliográfica resultaram 24 espécies de hidroides atecados já registradas, separados em 13 gêneros de 9 famílias. Das coletas do presente estudo foram identificadas 20 espécies de hidroides, agrupadas em 12 gêneros e 7 famílias, das quais 11 espécies são novas adições à fauna de hidroides atecados de Cuba. Como resultado deste estudo, a lista dos hidroides atecados de Cuba aumentou para 35 espécies separadas em 9 famílias e 18 gêneros. No recife de coral foram registradas 14 espécies, sendo *Eudendrium carneum* e *Coryne pusilla* as mais frequentes, e 7 apareceram nas gramas marinhas. *Myrionema amboinense* foi a espécie com maior frequência de ocorrência em gramas marinhas. *Rhizogeton conicum* foi a única espécie comum em ambos os habitats. Para testar se o gradiente de contaminação era um

impulsionador de variações na diversidade de hidroides atecados no recife de coral, diferentes contaminantes orgânicos químicos foram avaliados: esteróis (coprostanol, colesterol e estigmasterol), alquilbenzenos lineares (LABs), hidrocarbonetos aromáticos policíclicos (PAH), PAH de 4 a 6 anéis e hidrocarbonetos alifáticos (HA), que incluem a mistura complexa não resolvida (UCM). Nas gramas marinhas foram coletadas diferentes variáveis ambientais: profundidade, salinidade, turbidez, temperatura, pH, saturação de oxigênio (SO), oxigênio dissolvido (DO), demanda química de oxigênio (COD) e distância da costa (DistCoast) como indicadores de contaminação, baseado na qualidade da água e o impacto da contaminação da costa. No recife de coral de Havana, os melhores preditores para a riqueza de hidroides atecados foram coprostanol combinado com alquilbenzenos Linear Total (cop + LABs totais), e com a mistura complexa não resolvida (cop + UCM), mostrando a sensibilidade dos hidroides atecados a esses contaminantes. Nas gramas marinhas, a temperatura, salinidade, turbidez e a distância da costa marcaram a diferença de variação entre os locais. O oxigênio dissolvido também foi significativo para a variação nas assembleias de hidroides. Esses resultados mostram que a distribuição e diversidade das assembleias de hidroides atecados são variáveis sensíveis que responderam à variação de marcadores moleculares químicos indicadores de contaminação orgânica por esgoto, óleo e detergentes nos recifes de corais. Para a variação dos hidroides das pradarias de gramas, tiveram melhores respostas os fatores físicos das condições de qualidade da água como turbidez, distância da costa e oxigênio dissolvido, reforçando mais uma vez a importância deste grupo como indicadores ambientais.

**Palavras-chave:** Hidrozoários, Leptothecata, Anthoathecata, Gramas marinhas, Recifes de corais, diversidade, organic contamination, water quality.

## GENERAL ABSTRACT

Hydroids is the common name of the polypoid phase (benthic and asexual) of the hydrozoan life cycle. Class Hydrozoa and other classes of the Subphylum Medusozoa include, in the life cycle, an alternation of generations between the polypoid, benthic and asexual phase, and the medusoid, pelagic and sexual phase. This group of cnidarians is one of the most diverse in the consolidated benthic environment, with more than 3,200 species already described. The objective of this work was to carry out new surveys of the athecate hydroids, update the list of species of these organisms in Cuba, and evaluate their diversity and distribution in two shallow platform marine ecosystems, coral reefs and seagrass, as well as the relationship between the diversity of species and indicators of organic contamination in coral reefs and water quality indicators in seagrass. The collections were made in March/2013 and May/2011, respectively in coral reefs in northern Havana and in seagrass meadows, in Caibarien, Villa Clara. With the use of scuba diving, the collection techniques were visual censuses directed to scraping the hydroids along transections and indirect censuses of total scraping of the substrate organisms delimited by squares, where all the fauna and flora were collected for analyze the presence of epibiontic hydroids. As a result of the literature review, 24 species have already been registered, separated into 13 genera from 9 families. From the collections of the present study, 20 species of hydroids were identified, grouped into 12 genera and 7 families, of which 11 species are new additions to the fauna of athecate hydroids in Cuba. As a result of this study, the list of athecate hydroids in Cuba has been increased to 35 species separated into 9 families and 18 genera. On the coral reef, 14 species were recorded, with *Eudendrium carneum* and *Coryne pusilla* being the most frequent, and 7 species appeared on seagrasses. *Myrionema amboinense* was the species with the highest frequency of occurrence in seagrasses. *Rhizogetom conicum* was the only species common to both habitats. To test whether the contamination gradient was a driver of variations in the diversity of athecate hydroids in the coral reef, different organic chemical contaminants were used: sterols (coprostanol, cholesterol and stigmasterol), linear alkylbenzenes (LABs), polycyclic aromatic hydrocarbons (PAH), PAH of 4 to 6 rings and aliphatic hydrocarbons (HA) that include the unresolved complex mixture (UCM). Different environmental variables were collected from seagrasses: depth, salinity, turbidity, temperature, pH, oxygen saturation (SO), dissolved oxygen (DO), chemical oxygen demand (COD) and distance from the shore

(DistCoast) as indicators of contamination, based on water quality and the impact of coastal contamination. In the Havana coral reef, the best predictors for the richness of athecate hydroids were coprostanol combined with Total Linear alkylbenzenes (cop + total LABs), and with the unresolved complex mixture (cop + UCM) showing the sensitivity of the hydroids to these contaminants. In seagrass, temperature, salinity, turbidity, and distance from the coast marked the differences in variation between locations. Dissolved oxygen was also significant for the variation in hydroid assemblages. These results show that distribution and diversity of the athecate hydroid assemblages are sensitive variables that responded to the variation of chemical molecular markers that indicate organic contamination by sewage, oil and detergents in the coral reefs. For the variation of hydroids in seagrasses meadows, the physical factors of water quality conditions such as turbidity, distance from the coast and dissolved oxygen had better responses, reinforcing once again the importance of this group as environmental indicators.

Keywords: Hydrozoans, Leptothecata, "Anthoathecata", Marine seagrass, Coral reefs, diversity, organic contamination, water quality.

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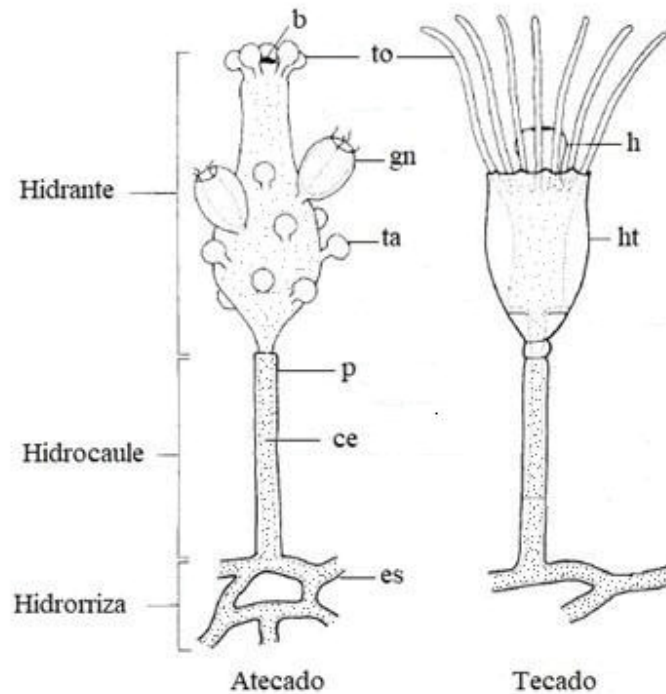
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## INTRODUÇÃO GERAL

Hidroide é o nome comum da fase polipoide das espécies da classe Hydrozoa, do Filo Cnidaria, subfilo Medusozoa. Em seu ciclo de vida, os Medusozoa apresentam uma alternância de gerações entre a fase polipoide, bentônica e assexuada, e a fase medusoide, pelágica e sexuada, que permanece fixa à estrutura reprodutora feminina em muitas espécies (Bouillon et al., 2004). Entre os Medusozoa, Hydrozoa é a classe com maior número de espécies e morfologicamente a mais diversa (Bouillon et al., 2006).

A forma básica do pólipo é cilíndrica, polarizada em um eixo oral-aboral, com a boca e os tentáculos na extremidade distal (superior) (Fig. 1; Schuchert, 2012). Os hidroides geralmente formam colônias (Bouillon et al., 2004). Nas colônias de hidrozoários, os pólipos compartilham uma cavidade gastrovascular interconectada mediante estolões tubulares, que formam a hidrorriza e o hidrocaule, exibindo variadas formas, desde colônias estolonais a verticais, ramificadas irregular ou regularmente, até colônias pelágicas (Bouillon et al., 2006; Cartwright & Nawrocki, 2010). Os tecidos da colônia de hidroides compõem os pólipos e conectam todas as partes da colônia formando o cenossarco. Um exoesqueleto quitinoso, chamado de perissarco, envolve o cenossarco (Genzano et al., 2014) e serve de proteção e suporte a colônia (Bouillon et al., 2006; Mendoza-Becerril et al., 2016).

FIGURA 1 – DIAGRAMA DOS HIDROIDES ATECADO E TECADO

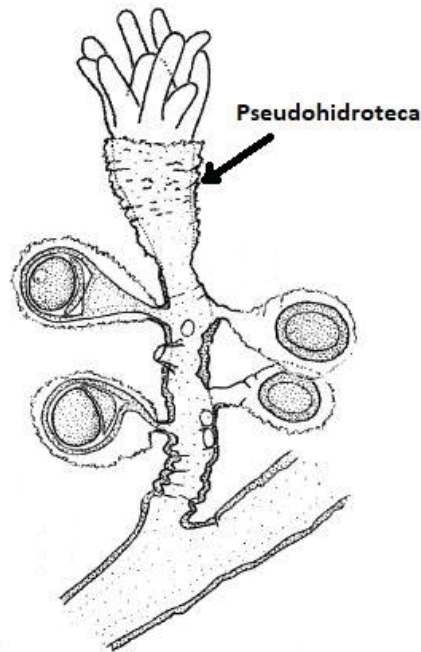


Fonte: Adaptado de Millard (1975).

(to: tentáculos orais b: boca; gn: gonóforo; ta: tentáculos aborais; p: perisarc; ce: cenosarco; es: estolão; h: hipostômio; ht: hidroteca).

Considerando os caracteres morfológicos, a maioria das espécies de hidroides é classificada em dois grupos: Leptothecata (tecados) e “Anthoathecata” (atecados). Os pólipos dos tecados estão recobertos por perissarco, desde a hidroriza até o hidrante, ao redor do qual forma uma estrutura chamada de hidroteca, e uma gonoteca que cobre os gonozoides (estrutura reprodutiva que produz os brotos de medusa). Os pólipos dos atecados não apresentam essa estrutura protetora ou apresentam o perissarco cobrindo apenas a parte basal dos pólipos (Allman 1872; Cornelius 1995; Genzano et al., 2014). Em alguns atecados o exoesqueleto pode ser reforçado por uma camada mais externa chamada de exosarco, que pode cobrir parte do hidrante, formando a pseudo-hidroteca (Mendoza-Becerril et al., 2016, 2018 a, Fig. 2). Muitos pólipos de tecados têm tecas muito reduzidas (*Hydranthea margarica* (Hincks, 1862)), ou às vezes não as têm (*Halecium lankesterii* (Bourne, 1890)), tornando o uso desse caráter para identificação nem sempre absoluto (Bouillon et al., 2006).

FIGURA 1 – DIAGRAMA DE HIDRANTE COM PSEUDO-HIDROTECA.



Fonte: Adaptado de Schuchert (2012).

O grupo Leptothecata é atualmente considerado monofilético, uma superordem (Maronna et al., 2016), enquanto o grupo “Anthoathecata” não é monofilético (Cartwright et al., 2008; Mendoza-Becerril et al., 2018a) e mostra a maior variabilidade exo-esquelética e complexidade estrutural entre os Hydrozoa, com destaque para as colônias pelágicas de Siphonophora (Mendoza-Becerril et al., 2016).

Vários tipos de tentáculos podem ser distinguidos entre os “Anthoathecata”, sendo os mais comuns os filiformes e os capitados. (Millard 1975; Bouillon et al., 2006; Bryant and Arehart, 2019). Os tipos de tentáculos e sua distribuição no corpo do hidrante constituem um importante caráter taxonômico (Schuchert, 2012).

A maioria dos hidroides é de pequeno tamanho e apresentam hábitos crípticos (Wedler and Larson, 1986). O número de caracteres morfológicos úteis para identificação é limitado e muitas espécies apresentam uma grande variação morfológica, tornando a taxonomia desses organismos confusa. Além disso, muitas espécies podem ser identificadas somente pela

morfologia das estruturas reprodutoras, muitas vezes ausentes nos espécimes coletados, ou do estágio da medusa (Calder and Cairns, 2009; Gili and Hughes, 1995). Em consequência, as variações morfológicas intraespecíficas frequentemente são mal interpretadas como variações interespecíficas ou vice-versa (Cunha et al., 2016). As características taxonômicas mais usadas para identificar os atecados são os tipos de nematocistos, a estrutura geral da colônia, a forma do pólip, o tipo e distribuição dos tentáculos e das estruturas reprodutivas (Millard, 1975; Bouillon et al., 2006).

Os nematocistos são organelas intracelulares produzidas pelos cnidócitos, utilizadas predominantemente para predação e defesa (Meier et al., 2012). Localizados principalmente nos tentáculos, os nematocistos constituem um caráter taxonômico exclusivo do filo Cnidaria (autapomorfia) (Bouillon, 2004; Beckmann and Özbek, 2012; Schuchert, 2012). Um nematocisto é uma cápsula que contém toxinas e uma estrutura tubular, enrolada em seu interior (Mariscal, 1974; David et al., 2008; Meier et al., 2012; Schuchert, 2012). A estrutura tubular tem morfologia variada, constituindo o caráter mais importante para a identificação dos tipos de nematocistos, junto com a forma e dimensões da cápsula. O diâmetro da estrutura tubular pode ser uniforme ou diferenciado, com dilatações e um filamento, e geralmente contém espinhos, em diferentes números, tamanho e disposição (Weill, 1929; Mariscal, 1974; Östman, 1979, 2000; Bouillon, 2004). Em uma espécie de Hydrozoa pode haver de um a vários tipos diferentes de nematocistos, que medem de 5 a 30  $\mu\text{m}$  nos “Anthoathecata” (Schuchert, 2012).

Hidrozoários são componentes das comunidades bentônicas de substratos duros e do zooplâncton gelatinoso (Gili e Hughes, 1995; Calder e Vervoort, 1998). São abundantes em todos os oceanos do mundo, desde os trópicos até as zonas polares e desde a zona entremarés até as grandes profundidades marinhas (Calder and Vervoort, 1998; Fernandez & Marques, 2018; Fernandez et al., 2020). Os hidroides têm ampla distribuição em todos os ecossistemas marinhos rasos, como costões rochosos, recifes de corais, manguezais, bancos de sargaço e gramas marinhas (Calder, 1995; Gili and Hughes, 1995). Muitas espécies são as primeiras a colonizar os substratos do bentos marinho e são importantes predadores de protozoários, bactérias, algas e crustáceos, entre outros organismos, além de sua dieta também pode conter matéria orgânica particulada (Gili and Coma, 1998; Bouillon et al., 2004, 2006). Dessa forma, participam da transferência de nutrientes e energia entre os ambientes bentônicos e pelágico,

conferindo-lhes grande importância ecológica nas cadeias tróficas marinhas (Gili and Hughes, 1995; Bouillon et al., 2004).

Apesar da grande importância dos hidroides no ambiente marinho, em Cuba esse grupo tem sido pouco estudado, embora tenha havido um incremento dos estudos nos últimos anos (Varela et al., 2005; Castellanos-Iglesias 2009; Varela e Cabrales, 2010, 2012; Varela, 2011; Castellanos-Iglesias et al., 2011, 2018). Quanto à diversidade e distribuição de hidroides em Cuba, o conhecimento é ainda menor, restringindo-se à um único artigo, Castellanos-Iglesias et al. (2018), que avalia a influência da poluição orgânica na distribuição, riqueza e abundância das comunidades hidroides tecados nos recifes de Havana.

Entre os ambientes marinhos costeiros de Cuba, os recifes de coral têm destacada importância ecológica e econômica e grandes extensões desses ecossistemas constituem áreas de preservação ambiental. Outro ecossistema costeiro amplamente distribuído na costa cubana são as pradarias de gramas marinhas, muitas vezes associadas a recifes de corais de áreas protegidas (e.g. ecossistema Sabana-Camangüey, na região norocidental de Cuba). Como habitat de hidroides, esses ecossistemas já foram tratados em vários estudos anteriores, tanto em gramas marinhas (Boero, 1981, 1987; Hughes et al., 1991; Kaehler and Hughes, 1992), quanto em recifes de corais (Gravier-bonnet and Bourmaud, 2006; Di Camillo et al., 2008; Castellanos-Iglesias et al., 2011, 2018; Mendoza-Becerril et al., 2018 b; Sayo, 2020), nos quais os hidroides tecados foram melhor representados do que os atecados.

Este estudo teve por objetivo avaliar a diversidade e distribuição dos hidroides atecados em dois ecossistemas marinhos rasos de Cuba, os recifes de corais de Norte Havana e gramas marinhas de Villa Clara, onde também foi avaliada a relação das espécies identificadas com indicadores de contaminação orgânica nos recifes e indicadores de qualidade da água em gramas marinhas. É o primeiro estudo direcionado a espécies de hidroides do grupo “Anthoathecata” nas águas da plataforma marinha norte e central de Cuba, excluindo as famílias Milleporidae Fleming, 1828 e Porpitidae Goldfuss, 1818, da subordem Capitata Kühn, 1913, e a família Stylasteridae Gray, 1847, da subordem Filifera Kühn, 1913 (classificação conforme WoRMS, em 29/3/2021). O estudo pretende ampliar o conhecimento da biodiversidade dos hidroides de Cuba. Este conhecimento constitui uma importante ferramenta de apoio à proteção e conservação de habitats marinhos no país, considerando ainda que o conhecimento das

espécies nativas é importante para reconhecer a introdução de espécies exóticas, bem como para a tomada de decisões referentes ao manejo das áreas a serem protegidas ou conservadas.

A dissertação está estruturada em dois capítulos, no formato de artigo para submissão em revistas científicas:

O capítulo 1 apresenta descrições das espécies de hidroides atecados (Cnidaria, Hydrozoa) coletadas em dois ambientes das águas rasas de Cuba, os recifes de corais de Havana e gramas marinhas de Villa Clara, e inclui também uma atualização da lista da fauna de hidroides atecados reportados para Cuba.

No capítulo 2 avalia-se a distribuição e a diversidade dos hidroides atecados de Cuba em habitats de recifes de corais e gramas marinhas, relacionado ao gradiente de contaminação nos recifes de coral de Havana e às condições de qualidade da água e impacto da contaminação no ecossistema de gramas marinhas em Villa Clara.



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## **CHAPTER 1**

### **NEW RECORDS, CONFIRMATIONS OF RECORDED SPECIES, AND UPDATE OF THE ATHECATE HYDROID FAUNA (HYDROZOA, “ANTHOATHECATA”) FROM SHALLOW- WATERS OF CUBA.**

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## **NEW RECORDS, CONFIRMATIONS OF RECORDED SPECIES, AND UPDATE OF THE ATHECATE HYDROID FAUNA (HYDROZOA, “ANTHOATHECATA”) FROM SHALLOW-WATERS OF CUBA.**

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### **Resumo**

Hidroides são hidrozoários muito comuns em ecossistemas marinhos rasos como recifes de corais, costões rochosos e gramas marinhas, e em diferentes latitudes e profundidades entre 0 e 3000 m. A maioria das espécies de hidroides estão classificados em dois grupos, Leptothecata (tecados) e “Anthoathecata” (atecados). Por se desenvolverem em diferentes habitats, é comum encontrar novos registros e novas espécies em águas cubanas, ainda pouco estudadas. Neste estudo, novos levantamentos de hidroides atecados foram realizados e foi atualizada a lista de espécies em Cuba. As coletas foram feitas em março/2013 e em maio/2011, em dois ecossistemas de águas rasas da plataforma marinha de Cuba, respectivamente em recifes de corais de norte Havana e pradarias de gramas marinhas em Caibarien, Villa Clara. Com o uso de “snorkeling” e mergulho autônomo, as técnicas de coleta foram censos visuais direcionados à raspagem dos hidroides ao longo de transecções e censos indiretos de raspagem total dos organismos de substratos delimitados por quadrados, onde toda a fauna e flora foram coletadas para analisar a presença de hidroides epibiontes. As amostras foram anestesiadas com mentol e fixadas em formalina 4%. Os hidroides foram separados dos substratos sob estéreo-microscópio e, para a identificação das espécies, foram preparadas

lâminas e fotografias em microscópios. As identificações foram embasadas principalmente nos estudos taxonômicos de Cuba, Caribe e região tropical do Atlântico Ocidental. Para confirmar as novas ocorrências, ampla literatura foi consultada. Da revisão bibliográfica resultaram 24 espécies de hidroides atecados já registradas, separados em 13 gêneros de 9 famílias. Das coletas do presente estudo foram identificadas 20 espécies de hidroides, agrupadas em 12 gêneros e 7 famílias, das quais 11 espécies são novas adições à fauna de hidroides atecados de Cuba. Dos 12 gêneros encontrados, 5 são novos para Cuba (*Cladocoryne*, *Slabberia*, *Pachycordyle Millardiana* e *Rhizogeton*), 8 foram encontrados em recifes de corais, 6 em gramas marinhas e 3 foram compartilhados pelos dois habitats. O gênero melhor representado foi *Eudendrium*, com 7 espécies, 5 delas em recifes de corais e 2 em gramas marinhas. Como resultado deste estudo, a lista dos hidroides atecados de Cuba aumentou para 35 espécies separadas em 9 famílias e 18 gêneros.

## Abstract

Hydroids are very common hydrozoan in marine ecosystems, such as coral reefs, rocky shores and sea grasses, occupying also niches in different latitudes and depths between 0 and 3000 m. Most species of hydroids are classified into two groups, Leptothecata (thecata) and “Anthoathecata” (athecata). As they develop in different habitats, it is common to find new records and new species in Cuban waters, yet little studied. In this study, new surveys were carried out, in the western and central regions of the country, and was updated the list of species of these organisms in Cuba. The collections were made in March/2013 and May/2011, in two shallow water ecosystems of the Cuban marine platform, respectively coral reefs in northern Havana and seagrass meadows in Caibarien, Villa Clara. With the use of “snorkeling” and scuba diving, the collection techniques were visual censuses directed to scraping the hydroids along transections and indirect censuses of total scraping of the substrate organisms delimited by squares, where all the fauna and flora were collected for analyze the presence of epibiont hydroids. The samples were anesthetized with menthol and fixed in 4% formalin. The hydroids were separated from the substrates under a stereomicroscope, and to identify the species slides were prepared to microscope observations and pictures. The identifications were based mainly on taxonomic studies in Cuba, Caribbean, and tropical region of the Western Atlantic. To confirm the new occurrences, extensive literature was consulted. The bibliographic review resulted in 24 species of athecate hydroids already registered, separated into 13 genera from 9 families. From the collections of the present study, 20 species of hydroids were identified, grouped into 12 genera and 7 families, of which 11 species are new additions to the fauna of athecate hydroids in Cuba. Of the 12 genera found, 5 are new records for Cuba (*Cladocoryne*, *Slabberia*, *Pachycordyle*, *Millardiana* and *Rhizogeton*). Eight were found on coral reefs, 6 on seagrass and 3 were shared by both habitats. The best represented genus was *Eudendrium*, with 7 species, 5 of them in coral reefs and 2 in seagrass. As a result of this study, the list of athecate hydroids in Cuba increased to 35 species separated into 9 families and 18 genera.

## 1. Introduction

The first collections of hydroids in Cuba were made during explorations in the Gulf Stream, between Florida and Cuba, by Mr de Pourtelés, assistant of United States Coast Survey. The species were later identified by Allman (1877), who report two species of thecate hydroids for Cuba (*Cryptolaria conferta* and *Plumularia macrotheca*). He refers that this region is characterized by a very distinct hydroid fauna, that should be part of a special province in the geographic distribution of hydroids. Later, Clarke (1879) published a study from the collections made by Agassiz during explorations in the Gulf Stream and in the Gulf of Mexico, with ten reports of hydroids to Cuba. Among these records of Cuba, the species *Pennaria disticha* Goldfuss, 1820 was described as a new species (as *P. symmetrica*), which was the only athecate reported to Cuba in the study of Clarke (1879).

Eight species of thecate hydroids and the species of athecate hydroid *Pennaria disticha* Goldfuss, 1820 as *Pennaria gibbosa* L. Agassiz, 1862 were reported for Cuban waters by Stechow (1912, 1914, 1919). Thirty species of thecate hydroids were report by Nutting (1985, 1900, 1904, 1915), and three species of thecate hydroids were reported by Fraser (1943), also for Cuban waters. According to a checklist of the hydroids of Gulf of Mexico published by Deveey (1954), 39 species of hydroids have been reported to Cuba, 38 of them belonging to the superorder Lepthothecata, only one, *Pennaria disticha*, to the "Anthoathecata".

New registers of hydroids to Cuba were published by Vervoort (1968) based on the collection of the Zoological Museum, Munich, Germany, (Zoologische Sammlung des Bayerischen Staates, München) from several expeditions to the Caribbean region. He also included a checklist of all hydroids reported up to that moment to the Caribbean, adding one more athecate species, *Turritopsis nutricula* (McCrady, 1857) (jellyfish form), reported by Mayer (1940) for Cuban waters.

In the first list of Cuban Cnidaria, Lalana (2001) reports 55 species of hydroids, of which only two are athecate: *Pennaria disticha* Clarck, 1879, as *Pennaria tiarella* (Ayres, 1854), and as *Halocordile disticha* (Goldfus, 1820), and two new records: *Bougainvillea niobe* Mayer, 1894 and an unidentified species of *Hydractinia*. The number of studies on the hydroid fauna of Cuba has increased recently with the publications of Castellanos Iglesias et al. (2006, 2009, 2011, 2018),

that added nine new records of athecates, and of Varela et al. (2005, 2011); Varela and Cabrales (2010, 2012), with eight new records of athecates, one of them a new species: *Zancklea cubensis* Varela, 2012. To date 25 species of athecate hydroids have been reported from Cuban waters, mainly from the western and central region of the country.

Hydroids are one of the most diverse group in the marine world, with more than 3729 species (Worms, visualized in 29/3/2021), and due to their ability to develop in various habitats, from shallow to deep water ecosystems, it is possible to continue to find new records and new species of this group in Cuban marine waters. In this way, the objective of this study was to carry out new surveys of athecate hydroids, in two shallow water zones in the north of Cuba, Havana coral reefs and the marine seagrass meadows in the city of Caibarien, Villa Clara, report and describe the new records and confirmations (species registered in this study that already were previously recorded in literature) and update the checklist of athecate hydroids for Cuba.

## **2. Materials and methods**

### **2.1. Study area**

The Cuban Island is located at the entrance to the Gulf of Mexico, south of Florida. It constitutes an archipelago composed of the big Island, the smaller Isla de la Juventud, and 1600 small islands all surrounded by the marine waters of the Caribbean Sea in the south, the waters of the Gulf of Mexico in the West and the Atlantic Ocean in the North.

The study area comprehends two shallow coastal areas in the north of the Cuban marine platform: coral reef of Havana and seagrass meadows in the city of Caibarien, province of Villa Clara. Hydroids were collected in May 2011 and March 2013, in fourteen sampling sites: seven on coral reefs and seven on seagrass meadows, in the western and north central regions of Cuba respectively. Study sites with locations are showed in Fig 1. and Table 1. For more details of the study areas see Castellanos-Iglesias, 2017).

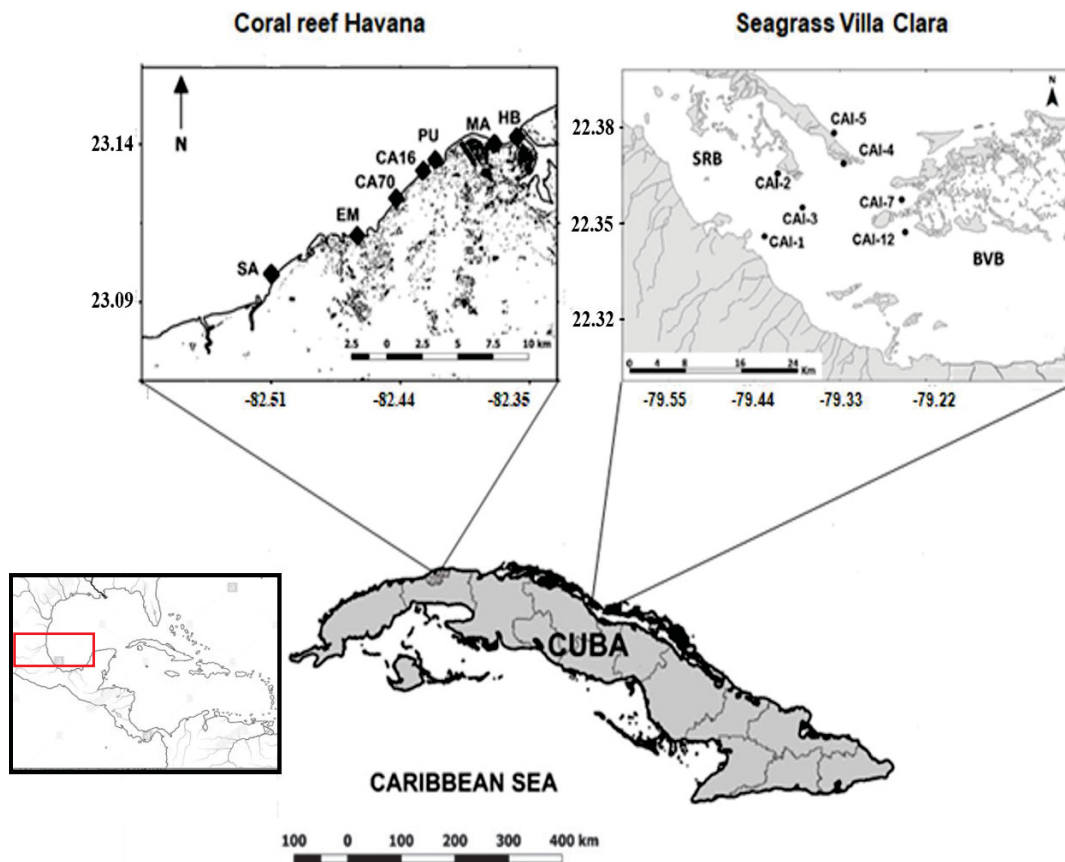


Figure 1. Maps of Central America and Caribbean and of Cuba with the location of the studied areas and sampling sites on coral reefs of Havana and seagrass meadows, Villa Clara. SRB= San Juan de los Remedios Bay; BVB= Buena vista Bay. CAI= Caibarien Municipality.

**Table 1. Station numbers (Stn.), study area, sampling sites and coordinates.**

Stn.	Study area	Sampling site	Lat (N)	Long (W)	Habitat
1	SRB	CAI-2	22 35.49	-79 23.98	SM
2	SRB	CAI-3	22 33.31	-79 22.04	SM
3	SRB	CAI-4	22 36.31	-79 19.30	SM
4	SRB	CAI-5	22 37.97	-79 19.74	SM
5	SRB	CAI-7	22 34.08	-79 15.04	SM
6	BVB	CAI-1	22 28.56	-79 16.08	SM
7	BVB	CAI-12	22 31.49	-79 14.46	SM
8	NH	HB	23 14 70	-82 35 86	CR
9	NH	MA	23 14 22	-82 37 00	CR
10	NH	ALM	23 13 69	-82 42 14	CR
11	NH	CA16	23 12 85	-82 42 31	CR
12	NH	CA70	23 12 56	-82 44 47	CR
13	NH	EM	23 10 64	-82 46 75	CR
14	NH	SA	23 09 33	-82 51 00	CR

**Legend: SRB- San Juan de los Remedios Bay, NH- North Havana; CAI- Caibarien Municipality; HB- Havana Bay; MA- Maceo; ALM- Almendares river; CA16- Calle 16; CA70- Calle 70; EM- Emisario; SA- Santa Ana, SM= Seagrass meadows; CR= Coral reefs.**

## 2.1. Data collection

Through a bibliographic survey, the records of the athecate hydroid for Cuba were verified with the purpose of updating the diversity and taxonomy of this group. The registers were compiled from documents in English and Spanish, including information on depth and collection sites, from both white literature (articles) and gray literature (academic documents). When necessary, more information about the study records was requested from the authors. Were also accessed online search tools like Google ([www.google.com](http://www.google.com)). The online searches were done through combined words, both in English and in Spanish (examples: hydroid and Cuba, Hydroid and Havana; hidroide and Cuba, Cnidaria and Cuba).

The new collections were made by SCUBA diving and snorkeling, using techniques of direct and indirect visual census. The current nomenclature of each species was verified using WoRMS 2020 and the available taxonomic literature.

### Coral reefs

In each of the seven sampling sites of the coral reefs of North Havana, three 10 m long transects were placed parallel to the coastline, at 10 meters depth. A direct visual census was made along each 10 m x 1 m transect for hydroids collection with their substrates. Following the transects six squares of 25 x 25 cm was positioned in each sampling site.

### Seagrass meadows

In seagrass meadows, two transects 30 m length perpendicular to each other, in the shape of a cross, were placed in each of the seven sampling sites. Along each transect, six quadrats of 25 x 25 cm were positioned 5 m distant from each other, summing a total of twelve sampling units per site. All fauna and flora within each quadrant in coral reefs and seagrass meadows were collected to analyze the presence of hydroids. Each sample were placed in plastic bags with local water, narcotized with menthol and fixed in 4% formalin.

#### *2.1. Laboratory procedures*

The sampled material was analyzed under a stereomicroscope Leica, the hydroids were separated from the substrates and morphotyped. Slides were prepared with fragments of the hydroid's colony or, for some morphotypes, the unique polyps. The material was analyzed and photographed in the Center for Advanced Fluorescence Technologies (CTAF) of the Setor de Ciências Biológicas da Universidade Federal de Paraná, in two optical microscopes: OLYMPUS-BX50 and AxioLab A1 ZEISS, both equipped with cameras Olympus DP 72 and AxioCam ERc 5s, respectively, linked to the imaging software ImageJ (Schneider et al., 2012) to perform the most acute measurements and identification. For each species was described the structures, the substrate when preserved and fertility, and measurements of the hydrocaulus, hydranth and nematocysts were added .

Cnidome (nematocysts) were examined mostly by compressing pieces of tissue or whole polyps between the slide and the coverslip (Calder, 1988). Nematocysts of each type were photographed and measured from each sample to determine the size range (length x diameter).



When it is possible at least 5 nematocysts of each type in each sample were measured. The categories of nematocysts were identified according to the classification of Mariscal (1974), Bouillon (2006) and Schuchert (2012).

For species identification we used mainly the taxonomic studies for Cuba, the Caribbean, and the tropical region of the Western Atlantic (Calder, 1988, 2010, 2013; Galea 2008, 2013; Puce et al., 2005a and 2005b). Taxonomic reports from other areas were also used (Kelmo and de la Santa-Isabel, 1998; Marques, 1993, 2000; Schuchert, 2001, 2004, 2005, 2006, 2007, 2008 a, b, 2009, 2012; Bouillon et al., 2004, De Vito et al., 2008). Although the classification of Cartwright et al., 2008, Maronna et al., 2016 and Mendoza-Becerril et al., 2018, in this study the classification and writing of the taxonomic terms were checked in the WoRMS - World Register of Marine Species (WoRMS, 2020).

### 3. Results

In the literature review, 24 species of athecate hydroids (Cnidaria, Hydrozoa) have already been registered in Cuban waters, separated into 13 genera and 9 families.

In the new surveys, 20 species of athecate hydroids were identified, belonging to seven families, and twelve genera (Table 2). Of the total species found, 18 was identified at a species level. Of the 20 species found in the two habitats, 14 were present in coral reefs and seven in seagrass meadows (table 2), only *Rhizogeton conicum* was shared in the two habitats. Of the eleven genera found, eight were present in coral reefs and six in seagrass meadows (table 2), of which three were shared by the two habitats *Eudendrium*, *Rhizogeton* and *Coryne*. The best represented genus was *Eudendrium*, with seven species, five of them in coral reefs and two in seagrass meadows.

As a result of this study, the checklist of the athecate hydroids increased to 35 species (Table 3), separated in nine families, and 18 genera.

**Table 2. List of hydroid species collected in 2011 and 2013 on the marine platform of northern and central Cuba (Coral reefs in Havana and seagrass meadows in Villa Clara). (Only hydroid form). Classification according to WoRMS (January 2021). NR: New record; CF: Confirmation, (\*): with reproductive structure.**

<b>Taxonomic report</b>	<b>NR/CF</b>	<b>Coral reef</b>	<b>Seagrass</b>
<b>Filo Cnidaria</b>			
<b>Clase Hydrozoa</b>			
<b>Subclase Hydroidolina</b>			
<b>Ordem "Anthoathecata"</b>			
<b>Suborder Capitata</b> Kühn, 1913			
Family Cladocorynidae Allman, 1872			
<i>Cladocoryne</i> Rotch, 1871	NR		
<i>Cladocoryne floccosa</i> Rotch, 1871	NR	X	
Family Corynidae Johnston, 1836			
<i>Coryne</i> Gaertner, 1774			
<i>Coryne pusilla</i> Alder, 1856	CF	X	
<i>Coryne</i> sp 1			X
<i>Slabberia</i> Forbes, 1846	NR		
<i>Slabberia strangulata</i> (McCrary, 1859)	NR	X	
Family Zancleidae Russell, 1953			
<i>Zanclea alba</i> (Meyen, 1834)	NR	X	
Family Pennariidae McCrary, 1859			
<i>Pennaria</i> Goldfuss, 1820			
<i>Pennaria disticha</i> Goldfuss, 1820 (*)	CF	X	
<b>Suborder Filifera</b> Kühn, 1913			
Family Bougainvilliidae Lütken, 1850			
<i>Millardiana</i> Wedler & Larson, 1986	NR		
<i>Millardiana longitentaculata</i> Wedler & Larson, 1986	NR		X
<i>Pachycordyle</i> Weismann, 1883	NR		
<i>Pachycordyle napolitana</i> Weismann, 1883	NR		X
Family Eudendriidae L. Agassiz, 1862			
<i>Eudendrium</i> Ehrenberg, 1834			
<i>Eudendrium capillare</i> Alder, 1856	CF	X	
<i>Eudendrium carneum</i> Clarke, 1882 (*)	CF	X	
<i>Eudendrium bermudense</i> Calder, 1988	NR	X	
<i>Eudendrium album</i> Nutting, 1898	NR	X	
<i>Eudendrium</i> sp.1			X
<i>Eudendrium klausii</i> Puce, Cerrano, Marques and Bavestrello, 2005	NR	X	
<i>Eudendrium moulouyensis</i> Marques, Peña Cantero and Vervoort, 2000 (*)	NR		X
<i>Myrionema</i> Pictet, 1893			
<i>Myrionema amboinense</i> Pictet, 1893 (*)	CF		X
Family Oceaniidae Eschscholtz, 1829			
<i>Corydendrium</i> Van Beneden, 1844			
<i>Corydendrium parasiticum</i> (Linnaeus, 1767)	CF	X	
<i>Rhizogeton</i> Agassiz, 1862	NR		
<i>Rhizogeton sterreri</i> (Calder, 1988)	NR	X	
<i>Rhizogeton conicum</i> Schuchert, 1996	NR	X	X
<i>Turritopsis</i> McCrary, 1857			
<i>Turritopsis nutricula</i> McCrary, 1857 (*)	CF	X	

**Table3. Updated checklist of the “Anthoathecata” hydroid species reported for Cuba. (Only hydroid form). Classification following world Register of marine species: WoRMS (visualized in January 2021).**

Check list	Locality	Latitude and Longitude	Depth	References
Hydrozoa				
Hydroidolina				
“Anthoathecata” Cornelius, 1992				
Aplanulata Collins, Winkelman, Hadrys & Schierwater, 2005				
Tubulariidae Goldfuss, 1818				
<i>Ectopleura</i> sp.	North Habana (Miramar)		12 m	Varela et al., 2005
<i>Ectopleura mayeri</i> Petersen, 1990	North Pinar del Río	22° 06' N 84° 50' W,	3 m	Varela et al., 2010
<i>Ralpharia gorgoniae</i> Petersen, 1990	Punta Francés, Isla de la Juventud	21° 30' 57 N 83° 10' 50 W	32 m	Castellanos-Iglesias et al., 2011
<i>Zyzyzus warreni</i> Calder, 1990	Los Cocos Key, Canarreos Archipelago	21° 56' 42 N 83° 22' 12 W	4 m	Castellanos-Iglesias et al., 2011
<i>Zyzyzus floridanus</i> Petersen, 1990	Bahía de Cochinos, provincia Matanzas			Varela et al., 2010
Capitata Kühn, 1913				
Cladocorynidae Allman, 1872				
<i>Pteroclava krempfi</i> (Billard, 1919)	North Havana		8 m	Varela, 2010
<i>Cladocoryne floccosa</i> Rotch, 1871	North Havana	231369 N -824.214 W	10 m	This study
Corynidae Johnstone, 1836				
<i>Coryne pusilla</i> Gaertner, 1774	North Havana	231470 N -823586 W 231256 N -824447 W 231285 N -824231 W 231064 N -824675 W 230933 N -825100 W	10 m	Castellanos-Iglesias et al., 2018, this study.
<i>Coryne eximia</i> Allman, 1859	North Havana		10m	Castellanos-Iglesias et al. 2018
<i>Coryne</i> sp.	El Holandés Península Guanahacabibes Pinar del Río, North Havana		2m	Varela, 2012, Castellanos-Iglesias et al., 2018
<i>Coryne</i> sp1	San Juan de los Remedios Bay (SRB)	22 37.97 N -79.19.74 W	2 m	This study

<i>Slabberia strangulata</i> (McCrady, 1859) Pennariidae McCrady, 1859	North Havana	23.1256 N -82.4447 W	10 m	This study
<i>Pennaria disticha</i> Goldfuss, 1820	Bahia Honda, Artemisa (north) North Havana	231470 N -823586 W 231285 N -824231 W 231256 N -824447 W	10 m	Clarke, 1879 as <i>Pennaria symmetrica</i> , Stechow, 1912 as <i>Pennaria gibbosa</i> L. Agassiz, 1862 Lalana et al., 2001 as <i>Pennaria tiarella</i> (Ayres, 1854) and as <i>Halocordile disticha</i> (Goldfuss, 1820); this study.
Sphaerocorynidae Prevot, 1959				
<i>Sphaerocoryne bedoti</i> Pictet, 1893	Punta Perdiz diving site at Cochinos Bay, Matanzas and Jardín de las Gorgonias, Guanahacabibes, Pinar del Río		5-15 m	Varela et al., 2010 Varela, 2012
Zancleidae Russell, 1953				
<i>Zanclea cubensis</i> (Varela, 2012)	North Havana		8 m	Varela, 2012
<i>Zanclea alba</i> Nutting, 1898	North Havana	231256 N -824447 W	10 m	This study
Filifera Kühn, 1913				
Eudendriidae Agassiz, 1862				
<i>Eudendrium bermudense</i> Calder, 1988	North Havana	231470 N -823586 W	10m	Castellanos-Iglesias et al., 2018, this study
<i>Eudendrium cf merulum</i> Watson, 1985	North Havana		10m	Castellanos-Iglesias et al., 2018.
<i>Eudendrium capillare</i>	North Havana	230933 N -825100 W	10 m	Castellanos-Iglesias et al., 2018; this study.
<i>Eudendrium carneum</i> Clarke, 1882	North Havana	23.1285 N -82.4231 W	10 m	Castellanos-Iglesias et al., 2018; this study.
<i>Eudendrium moulouyensis</i> Marques, Peña Cantero & Vervoort, 2000	North Havana	22 35.492 N -79 23.983 W 22 36.316 N -79 19.305 W 22 37.976 N -79 19.744 W 22 34.085 N -79 15.043 W 22 33.314 N -79 22.043 W 22 28.568 N -79 16.084 W 22 31.496 N -79 14.46 W	10 m	This study

<i>Eudendrium calceolatum</i> Motz-Kossowska, 1905	North Havana		10m	Castellanos-Iglesias et al., 2018
<i>Eudendrium album</i> Nutting, 1898	North Havana	231256 N -824447 W 231285 N -824231 W 231064 N -824675 W		This study
<i>Eudendrium</i> sp	North Habana (Miramar)		12 m	Varela et al., 2005
<i>Eudendrium</i> sp.1	San Juan de los Remedios Bay	22 33.314 N -79 22.043 W	2.5m	This study
<i>Eudendrium</i> sp.2	North Havana		10m	Castellanos-Iglesias et al., 2018
<i>Eudendrium</i> sp.3	North Havana		10 m	Castellanos-Iglesias et al., 2018
<i>Myrionema amboinense</i> Pictet, 1893	Matahambre-Matanzas (south) San Juan de los Remedios Bay Buena Vista Bay	22 35.492 N -79 23.983 W 22 36.316 N -79 19.305 W 22 37.976 N -79 19.744 W 22 34.085 N -79 15.043 W	1,5 m	Varela, 2012; this study.
<i>Hydractiniidae</i> L. Agassiz, 1862				
<i>Hydractinia</i> sp.	Playa Rosario, Golfo de Batabanó		2m	Ortiz, 2001; Lalana et al, 2001
<i>Oceaniidae</i> Eschscholtz, 1829				
<i>Corydendrium parasiticum</i> (Linnaeus, 1767)	North Habana (Miramar)	231369 N -824.214 W 231285 N -824231 W 231470 N -823586 W	12 m	Varela et al., 2005; this study.
<i>Turritopsis nutricula</i> McCrady, 1857	North Havana Bahía de Cochinos-Matanzas (south)	231285 N -824231 W 231064 N -824675 W	0,5-10 m	Varela et al., 2010; Castellanos-Iglesias, et al., 2018; this study.
<i>Rhizogeton sterreri</i> (Calder, 1988b)	North Havana	23.1369 N -82.4214 W	10 m	This study
<i>Rhizogeton conicum</i> Schuchert, 1996	Buena Vista Bay (BVB) Havana Bay	22 34.085 N -7915.043 W	3 m	This study
<i>Bougainvillidae</i> Lütken, 1850				
<i>Bougainvillia niobe</i> Mayer, 1894				Lalana et al. 2001

<i>Pachycordyle napolitana</i> Weismann, 1883	San Juan de los Remedios Bay (SRB)	22 37.976 N -79 19.744 W	2.8 m	This study
<i>Millardiana longitentaculata</i> Wedler & Larson, 1986	San Juan de los Remedios Bay (SRB)	22 35.492 N -79 23.983 W 22 34.085 N -79 15.043 W	2 -2.8 m	This study

## Order “Anthoathecata” Cornelius, 1992

### Suborder Capitata Kühn, 1913

### Family Cladocorynidae Allman, 1872

### Genus *Cladocoryne* Rotch, 1871

### *Cladocoryne floccosa* Rotch, 1871

(Fig. 2. A-F)

**Synonymy.** *Cladocoryne pelagica* Allman, 1876. Complete synonymy in Schuchert (2006).

**Material examined.** Stn. 10, March-2013, coral reef, 10 m deep, colony fragment with three polyps, up to 1.4 mm high, sterile, on algae *Lophophora* sp.

**Description.** Stolonal colony, with creeping hydrorhiza, Perisarc thin, with 4-5 annulations at the base of pedicels. Pedicels 595 µm in length, 129 µm in diameter. Hydranth cylindrical, 863 µm in length, and 240 µm in diameter, hypostome dome-shaped, 90 µm in length. Two types of tentacles: one oral whorl of six short capitate tentacles and approximately 10 aboral branched capitate tentacles in three whorls, Two series of nematocyst pouches, one between the base of the oral tentacles and the other between the proximal whorl of aboral tentacles.

**Cnidome (length x width).** Stenoteles in two sizes: small (5.7-6.9 x 4.2-5.3 µm), large (13.2-14.4 x 4.2-5.3 µm); in oral and aboral tentacles, hydranth body, and coenosarc of pedicels. Macrobasic euryteles: undischarged (23.7-27.5 x 9.9-12.4 µm), discharged (24.3-25 x 10.8-11.2 µm; shaft 54.7 55.5 µm), in hydranth body, and coenosarc.

**Remarks.** New record. According to WoRMS, there are five accepted species of the genus *Cladocoryne*: *C. floccosa* Rotch, 1871; *C. haddoni* Kirkpatrick, 1890; *C. littoralis* (Mammen,

1963); *C. minuta* Watson, 2005; *C. travancorensis* (Mammen, 1963), of which only *C. floccosa* reported for the North Atlantic and Caribe. The polyps of our sample have six oral tentacles differing from *C. littoralis*, *C. minuta*, and *C. travancorensis* with four, and, also differing of *C. travancorensis* which have aboral tentacles septated. *C. haddoni* has two whorls of aboral branched capitate tentacles, two sizes of macrobasic euryteles, and there is not stenoteles in its cnidoma. The size of the macrobasic euryteles of our specimen is smaller than those reported by Millard (1975), Schuchert (1996) and Migotto (1996). The size of the stenoteles is like those reported by Millard (1975).

**Great Caribbean records.** Gulf-stream (Allman 1876 as *C. pelagica*); Tortuga Island (Fraser 1947 as *C. pelagica* Allman 1876); Puerto Rico (Wedler and Larson, 1986); Guadeloupe and Martinique (Galea, 2008, 2013); Atlantic coast of Florida (Calder, 2013).

**Geographical distribution.** Circumglobal in temperate to tropical waters (Millard, 1975; Schuchert, 2006), occasionally extending into temperate areas (Millard, 1975).

### **Family Corynidae Johnston, 1836**

#### **Genus *Coryne* Gaertner, 1774**

#### ***Coryne pusilla* Gaertner, 1774**

(Fig 3. A-E)

**Synonymy.** Complete synonymy in Schuchert (2001).

**Material examined.** Stn. 11, March-2013, coral reef, 10 m deep, stolonial colony fragment with one polyp, up to 3.1 mm high, sterile on *Sargassum* sp. Stn. 13, March-2013, coral reef, 10 m deep, colony fragment with stolonial polyps up to 1.8 mm high, fertile, on *Sargassum* sp. Stn. 11, March-2013, coral reef, 10 m deep, colony fragment with two polyps, up to 2.7 mm high, fertile, on *Sargassum* sp. Stn. 11, March-2013, coral reef, 10 m deep, branched colony fragments, up to 2.6 mm high, fertile, on dead octocoral. Stn. 11, March-2013, coral reef, 10 m deep, four colony fragments with five polyps, sterile, on *Dictyota* sp. Stn. 11, March-2013, coral reef, 10 m deep, stolonial colony, up to 3,7 mm high, fertile. Stn. 12, March-2013, coral reef, 10 m deep,

stolonial colony fragment, only one polyp, up to 2.5 mm high, on *Halimeda* sp. Stn. 14, March-2013, coral reef, 10 m deep, one stolonial polyp, sterile, on *Sargassum* sp.

**Description.** Stolonial or branched colonies with creeping hydrorhiza. Pedicels 0.35 -2 mm in length, with 4-6 annulations or wrinkles at the base and a few others irregularly distributed along the pedicel, measuring 134-217  $\mu\text{m}$  in diameter in the distal part, and 93–119  $\mu\text{m}$  at the basal part. Hydranth total length 591-1098  $\mu\text{m}$ , and 124-271  $\mu\text{m}$  in diameter, cylindrical in relaxed polyps to spindle shape in contracted polyps. Short hypostome, rounded to conical, 101-196  $\mu\text{m}$  in length. All tentacles are capitated. One oral whorl of 4-5 tentacles, and up to 25 aboral tentacles scattered in the hydrant body. Spherical gonophores, as fixed sporosacs, born between the aboral tentacles, approximately in the lower half of the hydrant's body; up to 4 gonophores per polyp.

**Cnidome (length x width).** Stenoteles in two sizes: small (6.3-12.1 x 4.5-7.7  $\mu\text{m}$ ), large (16.7-21.6 x 12-16.1  $\mu\text{m}$ ), in capitate tentacles and in coenosarc of hydrocaulus.

**Remarks.** Three species of the genus *Coryne* have been reported for the Caribbean, *C. pusilla*, *C. eximia*, and *C. sargassicola*. *C. sargassicola* has four filiform aboral tentacles, which may be absent, and has desmoneme in the cnidome. The colonies under study do not have filiform tentacles or desmoneme and have fixed gonophores as sporosacs, like that described by Schuchert (2001), for *C. pusilla*, and unlike the *C. eximia* gonophores as medusa buds.

**Great Caribbean records.** Guadeloupe (Galea, 2008), Cuba (whithout description) (Castellanos-Iglesias et al., 2018).

**Geographical distribution.** Circumglobal (Schuchert, 2005).

### ***Coryne* sp.1**

(Fig 4. A-E)

**Material examined.** Stn. 4, May 2013, seagrass, 2 m deep, one small polyp, without gonophores, on *Thalassia testudinum*. Stn. 4, May 2013, seagrass, 2 m deep, colony fragment, fertile, on *Thalassia testudinum*.



**Description.** Stolonal colonies, up to 2.1 mm high, with creeping hydrorhiza. Pedicels 900 µm in length, annulations throughout the pedicel, dilatation like funnel (perisarc collar) in distal end in one of the polyps. Hydranth total length 1.4 mm, and 221 µm in diameter, spindle shaped. Short and rounded hypostome, 291 µm in length. All tentacles capitate. One oral whorl of 4-5 tentacles, and approximately 27 aboral tentacles scattered in the hydrant body. Spherical gonophores, 299 µm length and 232 µm wide, born between the aboral tentacles, in the lower half of the hydrant's body, up to 3 gonophores per polyp.

**Cnidome (length x width).** Stenoteles in two sizes: large (18.8-25.7 x 13.5-16 µm), small stenoteles (11.6-15.1 x 7.4-8.7 µm).

**Remarks.** A funnel shaped expansion (perisarc collar) at the distal part of the perisarc was described by Schuchert (2001) for *C. pusilla*, *C. eximia* and *C. muscoides*. Of those *C. pusilla* and *C. eximia* have been reported for the Caribbean region. *C. eximia* have gonophores as medusa buds, *C. pusilla* and *C. muscoides* have it as fixed sporosacs. Our material has very immature gonophores, so it is difficult to define if they are fixed gonophores or young medusa buds. For this reason, it was decided to nominate it as *Coryne* sp.1.

### **Genus *Slabberia* Forbes, 1846**

#### ***Slabberia strangulata* (McCraday, 1859)**

(Fig 5. A-D)

**Synonymy.** Complete synonymy in Schuchert (2001).

**Material examined.** Stn. 12, March 2013, coral reef, 10 m deep, one stolonal polyp, up to 1.08 mm, sterile, on algae.

**Description.** Stolonal colony. Perisarc thin, without annulations, covering stolon to the base of hydranth, pedicel with 63-67 µm wide. Hydranth fusiform, orange in color, with 570 µm in total length and 147 µm in diameter, hydrant body length 421 µm, Rounded and short hypostome, 150 µm. in length. One oral whorl of 4 capitated tentacles and 4 filiform aboral tentacles at lower third of the hydranth.

**Cnidome (length x width).** Stenoteles in two size: small (11.7-12.9 x 7-8  $\mu\text{m}$ ), large, undischarged (22.8-30.6 x 13.3-16.7  $\mu\text{m}$ ), discharged (26.8 x 14.9  $\mu\text{m}$ , shaft 22  $\mu\text{m}$ ), abundant in capitate tentacles and scattered at the hydrant body and coenosarc.

**Remarks.** There are five species of Corynidae accepted in WoRMS that have hydranths with one oral whorl of capitate tentacles and one aboral whorl of filiform tentacles as explained by Calder (1970) and Schuchert (2001): *Cladonema radiatum* Dujardin, 1843, *Slabberia strangulata* (McCrady, 1859) as *Dipurena strangulata* McCrady, 1859, *Stauridiosarsia reesi* (Vannucci, 1956) as *Dipurena reesi* Vannucci, 1956, *Stauridiosarsia spongicola* (Anger, 1972) as *Dipurena spongicola* Anger, 1972, and *Sarsia piriforma* Edwards, 1983. Our material is composed of a single polyp with contracted tentacles, so it has been impossible to observe the number of endodermal cells, and the form of the knob of the capitate tentacles, that differentiate these species.

*Sarsia piriforma* have the filiform aboral tentacles around the middle of the hydranth body (Schuchert, 2001), as *Dipurena reesi* (Brinckman and Petersen, 1960; and Calder, 1970), Our specimen has the filiform tentacles at the lower third of hydranth. *Stauridiosarsia spongicola* has 5-7 filiform tentacles unlike our material that has four, and, in addition to the stenoteles, *S. spongicola* has very variable isorhizas (Schuchert, 2001). *Cladonema radiatum* also has macrobasic euryteles in addition to the stenoteles (Brinckman and Petersen, 1960). Our material has only stenoteles with form and sizes similar to those mentioned for *Slabberia strangulata* (as *Dipurena strangulata*) by Calder (1970) and Schuchert (2001). For this reason, and due to its distribution close to the study area, it was decided to identify our material as *Slabberia strangulata*. However, samples with gonophores are needed for more precise identification.

**Great Caribbean records.** Puerto Rico (Schuchert, 2001).

**Geographical distribution.** USA, Puerto Rico, and Gulf of Guinea (Schuchert, 2001).

### Family Zancleidae

#### Genus *Zanclea* Gegenbaur, 1856

#### *Zanclea alba* (Meyen, 1834)

(Fig 6. A-B)

**Synonymy.** Complete synonymy in Calder (1988)

**Material examined.** Stn. 11, March-2013, coral reef, 10 m deep, stolonial colony fragment with three polyps, sterile, on *Sargassum* sp. Stn. 11, March-2013, coral reef, 10 m deep, colony fragment with five polyps, up to 2.6 mm, sterile.

**Description.** Stolonial or branched colonies, with creeping hydrorhiza. Perisarc thin, terminating at hydranth base. Pedicels 485- 802  $\mu$ m in length, annulations or wrinkles at the base, measuring 65-68  $\mu$ m in diameter, expanding distally 132-151  $\mu$ m. Hydranth cylindrical, elongated 1-1.9 mm in total length, and 165-178  $\mu$ m in diameter, elongated cylindrical shape Hypostome short and dome shaped. Approximately 50 capitated tentacles, one oral whorl of 6 tentacles, aboral tentacles scattered in the hydrant body.

**Cnidome (length x width).** Stenoteles in two sizes, in tentacles capitulation. Nematocysts could not be measured.

**Remarks.** According to Shuchert (2010) the occurrence in sargassum and the absence of macrobasic euryteles in the polyp are diagnostic characters of the species *Z. alba*.

**Great Caribbean records.** Colombia (Fraser, 1947, as *Z. costata*); Belize (Spracklin, 1982, as *Z. costata*; Calder, 1991 a, b); Puerto Rico (Wedler and Larson, 1986, as *Zanclaea* sp.).

**Geographical distribution.** Apparently circumglobal in tropical and temperate waters (Calder, 1988).

### **Family Pennariidae McCrady, 1859**

#### **Genus *Pennaria* Goldfuss, 1820**

#### ***Pennaria disticha* Goldfuss, 1820**

(Fig 7. A-H)

**Synonymy.** Complete synonymy in Calder (1988) and Schuchert (2006).

**Material examined.** Stn. 12, March 2013, coral reef, 10 m deep, colony fragment, fertile. Stn. 1, coral reef, 10 m deep, colony fragment. Stn. 12, March 2013, coral reef, 10 m deep, colony

fragment. Stn. 12, March 2013, coral reef, 10 m deep, colony fragment with five polyps, up to 1 cm high. Stn. 8, March 2013, coral reef, 10 m deep, colony fragment. Stn. 11, coral reef, 10 m deep, colony fragment. Stn. 11, March 2013, coral reef, 10 m deep, colony fragment. Stn. 12, March 2013, coral reef, 10 m deep, colony fragment, sterile. Stn. 8, March 2013, coral reef, 10 m deep, colony fragment up to 1.6 cm, sterile.

**Description.** Large, pinnate, erect colony fragments. Alternating branches, monopodial growth, with terminal hydrants, arising from a creeping hydrorhiza, Thick hydrocaulus, with 164-172  $\mu\text{m}$  in diameter. Perisarc thick and smooth, yellow to dark brown, becoming clearer from the base to distal parts of hydrocaulus and hydrocladium. Groups of 3-4 annulations at regular intervals along hydrocaulus, usually above the insertion of the pedicels, and from 6-7 annulations at the base of pedicels. Spindle to pear-shaped hydranth, 1.1 mm in total length, hydrant body length 620  $\mu\text{m}$ , and 458  $\mu\text{m}$  in diameter. Large hypostome, dome-shaped, 444  $\mu\text{m}$  in length. Two types of tentacles: 14-16 short and capitated tentacles distributed in three whorls in the distal half of the hydrant and one aboral whorl of 12-14 long and slender filiform tentacles. Gonophores present as oblong medusoid, 857  $\mu\text{m}$  in length and 675  $\mu\text{m}$  in wide, arising in short pedicels above the whorl of aboral filiform tentacles.

**Cnidome (length x width).** Stenoteles of three sizes: large undischarged (24.7-27.6 x 15.5-17.8)  $\mu\text{m}$ , discharged (31 x 14  $\mu\text{m}$ , shaft 23.3  $\mu\text{m}$ ); medium undischarged (15.9 x 10.6)  $\mu\text{m}$ , small undischarged (5.8-7.7 x 3.6-6.1)  $\mu\text{m}$ ; microbasic b mastigophore with a basal, spherical inclusion (10.2-12.8 x 5.6-8.1)  $\mu\text{m}$ , desmoneme (4.4 x 2.6)  $\mu\text{m}$ , basitrichous isorhiza (5.9-6.4 x 2.2-2.4)  $\mu\text{m}$ .

**Remarks.** Confirmation. *Pennaria disticha* has a wide distribution in the Caribbean (Calder and Kirkendale, 2005). It is the only species of the genus *Pennaria* reported for Cuba and for the Caribbean. According to molecular studies, *Pennaria disticha* is currently considered as a complex of cryptic species, with four lineages for the Atlantic Ocean (Miglietta et al 2015, 2019).

**Great Caribbean records.** Cuba, Clarke 1879, as *Pennaria symmetrica* n. sp.; Charlotte Amalia, St. Thomas, Stechow 1919, as *Pennaria tiarella* (Ayres 1852); Bermudas, Calder 1988, as *Halocordyle disticha* (Goldfuss, 1820); Costa Rica, Caribbean coast, Kelmo and Vargas 2002; Guadeloupe, Galea 2008; Gulf of Mexico (Calder & Cairns 2009); Martinique, Galea 2013;

Kingston, Jamaica, Kükenthal & Hartmeyer, 1907; St. Thomas, Sound, Kükenthal & Hartmeyer, 1907; Barbados, Kükenthal & Hartmeyer, 1907; La Guaira, Venezuela, E. Hentschel, 1922; Puerto Cabello, Venezuela, E. Hentschel, 1923; Colon, Panama, E. Hentschel, 1932; Limon, Costa Rica, E. Hentschel, 1922) in Vervoort 1968;

**Geographical distribution.** Circumglobal in warm temperate to tropical waters (Schuchert 2006, Calder 2010).

**Suborder Filifera Kühn, 1913**

**Family Bougainvilliidae Lütken, 1850**

**Genus *Pachycordyle* Weismann, 1883**

***Pachycordyle napolitana* Weismann, 1883**

(Fig 8. A-F)

**Synonymy.** Complete synonymy in Schuchert (2004).

**Material examined.** Stn. 5, May 2013, seagrass, 2 m deep, colony fragment, up to 4.4 mm, sterile, on *Thalassia testudinum*.

**Description.** Colony fragment, with creeping hydrorhiza. With two pedicels without hydrants and two complete polyps. Perisarc irregularly and scarcely corrugated throughout, not distinctly double-layered, terminating at base of hydranth, pseudohydrotheca absent. Pedicel length 1.23 mm with three annulations at the base. Hydranth club or barrel-shaped with 1.1 mm in total length, hydranth body length 879  $\mu\text{m}$ , and 405  $\mu\text{m}$  in diameter, hypostome not visualized. Filiform tentacles approximately 18 in number, in two or more whorls around the hypostome.

**Cnidome.** Small heterotrichous microbasic euryteles undischarged (7.5-9.3 x 3.9-4.4  $\mu\text{m}$ ), discharged (7.2-8.6 x 3.4-5  $\mu\text{m}$ , shaft 5.3-7.5  $\mu\text{m}$ ), abundant on tentacles, hydranth body and coenosarc. Desmoneme (5-6 x 3.2-3.5  $\mu\text{m}$ ), on tentacles, and hydranth body.

**Remarks.** Our material is like that described by Calder (1988) for Bermudas. Samples with gonophores are needed for more precise identification.

**Great Caribbean records.** Puerto Rico (Wedler and Larson, 1986); Bermuda (Calder, 1988); Belize (Calder, 1991).

**Geographical distribution.** See specific records in Schuchert (2004).

**Genus *Millardiana* Wedler & Larson, 1986**

***Millardiana longitentaculata* Wedler & Larson, 1986**

(Fig 9. A-C)

**Material examined.** Stn. 5, May 2013, seagrass, 2.5 m deep, sample colony up to 2.7 mm, with two polyps, sterile, on *Thalassia testudinum*. Stn.1, May 2013, seagrass, 2.5 m deep, sample colony up to 1.4 mm, with one polyp, sterile, on *Thalassia testudinum*.

**Description.** Stolonal hydranth, red in color. Hydranth fusiform with prominent cylindrical hypostome. Thin perisarc, terminating at the base of hydranth, pseudohydrotheca absent. Pedicel length 336-1010  $\mu\text{m}$ , and 312-392  $\mu\text{m}$  in diameter. Hydranth fusiform 1.1 mm in total length, hydrant body length 717-733  $\mu\text{m}$ , hydranth diameter 312-392  $\mu\text{m}$ , hypostome prominent 394-421  $\mu\text{m}$  in length. Long and filiform 13-16 tentacles, arranged in 2-3 close whorls below the hypostome.

**Cnidome (length x width).** Small heterotrichous microbasic euryteles (7.5-10 x 2.5-3.4  $\mu\text{m}$ ) and desmonemes (5.2-5.8 x 3-3.5  $\mu\text{m}$ ); abundant on tentacles, hydranth body and coenosarc.

**Remarks.** New record. *Millardiana* is a monotypic genus, distinguished by the red pigmentation of the hydrants, the extremely long tentacles and prominent hypostome (Calder 1988).

**Great Caribbean records.** Puerto Rico (Wedler and Larson, 1986); Bermudas (Calder, 1988); Belize (Calder, 1991); Guadeloupe (Galea, 2008).

**Geographical distribution.** Only Caribbean records.

**Family Eudendriidae L. Agassiz, 1862**

**Genus *Eudendrium* Ehrenberg, 1834**

***Eudendrium capillare* Alder, 1856**

(Fig 10. A-C)

**Synonymy.** Complete synonymy in Calder (1988).

**Material examined.** Stn. 14. March-2013, coral reef, 10 m deep. Colony fragment with 3 polyps, up to 4 mm, sterile, on calcareous algae. Stn. 14, March-2013, coral reef, 10 m deep. only one polyp, sterile, on calcareous algae.

**Description.** Small and stolonal colony, with creeping hydrorhiza. Stolon and base of pedicel yellow, each pedicel with 5 to 7 annulations at base, and one group of 3-4 in the middle. long pedicels, with 1.5-2.7 mm in length, and 97-129  $\mu\text{m}$  in wide. hydranth total length 470-569  $\mu\text{m}$ , hydranth body length 327-392  $\mu\text{m}$ , hydranth diameter 318-715  $\mu\text{m}$ , hypostome trumpet shaped, 143-177  $\mu\text{m}$  in length. Tentacles solid and filiform, 15-20, in one whorl.

**Cnidome (length x width).** Only small heterotrichous microbasic euryteles (6.8-7.7 x 2.8-4  $\mu\text{m}$ ), on tentacles, hydranth, and coenosarc.

**Remarks.** Confirmation. According to Schuchert (2008 b), in some colonies, the presence of complementary isorhizas. with a length like that of euryteles has been observed. In our sample we did not find isorhizas.

**Great Caribbean records.** Puerto Rico (Fraser, 1944); Texas, U.S.A (Deevey, 1950). Bermudas (Calder, 1988); Panamá (Calder, 2005); Guadeloupe (Galea, 2008); Gulf of México and Yucatán (Calder and Cairns, 2009) as *Eudendrium tenue* A. Agassiz, 1865; Cuba, (without description) (Castellanos-Iglesias et al., 2018).

**Geographical distribution.** Possibly cosmopolitan, (Marques et al., 2000). Complete registers in Calder (1988); Marques (2001); Mendoza-Becerril (2020).

***Eudendrium carneum* Clarke, 1882**

(Fig 11. A-F)

**Synonymy.** *Eudendrium cunninghami* Kirkpatrick, 1910. *Eudendrium ramosum* McCrady, 1859; A. Agassiz, 1865; Congdon, 1906; Fraser, 1912; Bennitt, 1922.



**Material examined.** Stn. 13, March-2013, Coral reef, 10 m deep, one hydrocaulus with one polyp. Stn. 13, March-2013, Coral reef, 10 m deep, on octocoral. Stn. 11, March-2013, Coral reef, 10 m deep, one hydrocaulus, with 3 polyps, on bivalve. Stn. 11, March-2013, Coral reef, 10 m deep, colony fragments, up to 2,5 cm, fertile. Stn. 14, March-2013, Coral reef, 10 m deep, colony up to 2 cm high, fertile, on octocoral. Stn. 14, March-2013, Coral reef, 10 m deep, colony fragment with three stolonal polyps up to 3.7 mm high, sterile. Stn. 12, March-2013, Coral reef, 10 m deep, colony up to 2 cm high, on *Halimeda* sp. Stn. 14, March-2013, Coral reef, 10 m deep, colony up to 2.5 mm high. Stn. 11, March-2013, Coral reef, 10 m deep, colony up to 2.2 cm, fertile. Stn. 9 March-2013, Coral reef, 10 m deep, colony up to 5 cm, sterile. Stn. 11, March-2013, Coral reef, 10 m deep, sterile. Stn. 11, March-2013, Coral reef, 10 m deep, three hydrocauli, up to 1.7 cm, sterile. Stn. 11, March-2013, Coral reef, 10 m deep, fertile. Stn. 11, March-2013, Coral reef, 10 m deep, colony fragment, up to 2.6 cm, sterile. Stn. 12, March-2013, Coral reef, 10 m deep, colony fragment with two polyps, sterile. Stn. 12, March-2013, Coral reef, 10 m deep, branched colony, up to 6 cm high, fertile. Stn. 12, March-2013, Coral reef, 10 m deep, branched colony, sterile. Stn. 12, March-2013, Coral reef, 10 m deep, branched colony, up to 1,5 cm. Stn. 12, March-2013, Coral reef, 10 m deep, four colony fragments, up to 2.5 cm, fertile, on calcareous algae.

**Description.** Colonies of two types, stolonal and branched, some slightly fascicled. Branching alternated up to third order considering pedicels of gonophores. Hydrocaulus brown, yellow to transparent in the distal part of branches. Annulations: 3-4 at the base of pedicels and branches, with occasional annulations along the hydrocaulus and pedicels. Diameter of pedicel 115-187  $\mu\text{m}$ . Tentacles solid and filiform, in one whorl below hypostome, 14 to 22 in number. Hydranth urn-shaped, 396-934  $\mu\text{m}$  in total length, hydranth body length 161-566  $\mu\text{m}$ , hypostome trumpet shaped, 136-424  $\mu\text{m}$  length. Groove at the base of hydranth. Female gonophores with 2-8 eggs, spadix bifid and curved over the egg, arising from the hydranth body under degenerating tentacles, eggs scattered over the pedicel when mature with total tentacles reduction, and thickened by a perisarc capsule.

**Cnidome (length x width).** Small heterotrichous microbasic eurytheles (6.6-9.3 x 2.9-5  $\mu\text{m}$ ), in tentacles and coenosarc; heterotrichous anisorhizas, undischarged (18.5-26.3 x 7.6-12.5  $\mu\text{m}$ ),



discharged heterotrichous anisorhizas (18.2 x 7.2  $\mu\text{m}$ , shaft 14.5  $\mu\text{m}$ ), in hydranth body, hypostome, coenosarc and gonophores.

**Remarks.** Confirmation. According to Marques (2001) the cnidoma (large heterotrichous anisorhizas (with thread slightly dilated toward base and spines larger at base of thread, Bouillon et al. 2004) and the morphology of the gonophores are considered diagnostic characters for *E. carneum*.

**Great Caribbean records.** Bermudas (Calder, 1988); Costa Rica, Limon, (Kelmo et al., 2002); Gulf of México and Yucatán (Calder and Cairns, 2009); Panama (Calder, 2005); Puerto Rico, Culebra Island (Wedler and Larson, 1986); EE.UU (Calder, 2013); Cuba (without description), (Castellanos-Iglesias, et al., 2018).

**Geographical distribution.** Complete registers in Marques (2001) and Schuchert (2008 b).

### ***Eudendrium klausi* Puce, Cerrano, Marques and Bavestrello, 2005**

(Fig 12. A-D)

**Material examined.** Stn. 11, March-2013, coral reef, 10 m deep, colony up to 1.27 cm, few branches up to 1<sup>st</sup> order, sterile, on octocoral. Stn. 12, March-2013, coral reef, 10 m deep, colony up to 1.5 cm high, branched up to 2<sup>nd</sup> order, sterile.

**Description.** Colony unfascicled, alternated branched. Slightly annulated: 3-4 at base of hydrocaulus, 2-4 at base of pedicels. Pedicel's length 1.8-3.5 mm in length and 140-172  $\mu\text{m}$  wide. Hydranth total length 734  $\mu\text{m}$ -1.2 mm, hydranth body length 427-761  $\mu\text{m}$ , hydranth diameter 444-889  $\mu\text{m}$ , hypostome large and flared, 306-464  $\mu\text{m}$  in length. Approximately 23-28 tentacles, in one whorl below hypostome. Abundant granules of blue pigment along the hydranth body, hypostome, tentacles and coenosarc. Spiny aspect of tentacles due to nematocysts distribution.

**Cnidome (length x width).** Nematocysts of one category, heterotrichous microbasic euryteles of two size-classes: small (7.1 – 8.6 x 3.3 – 4.7  $\mu\text{m}$ ), in tentacles, hydranth body and hypostome; largenot discharged )18-24.8 x 8.3-12  $\mu\text{m}$ ), discharged (17.5-21.4 x 9-11.5  $\mu\text{m}$ ); discharged shaft 11.8-17.3  $\mu\text{m}$  in length). in the hydrant body, hypostome and coenosarc.

**Remarks.** New record. In our material, the hydrocaulus have alternated branches unlike the description by Puce et al (2005) that is irregular. Our material differs from other species of the genus *Eudendrium* with similar cnidome by the presence of abundant granules of blue pigment in the hydranth body, hypostome, tentacles and coenosarc, and by the spiny aspect of tentacles, as it was summarized in Puce et al. (2005).

**Great Caribbean records.** Only recorded in Belize (Puce et al., 2005).

**Geographical Distribution.** Only Caribbean records.

### ***Eudendrium moulouyensis* Marques, Peña Cantero and Vervoort, 2000**

(Fig 13. A-E)

**Material examined.** Stn. 1, May 2013, seagrass, 2 m deep, colony fragment with four polyps, sterile, on *Thalassia testudinum*. Stn. 1, May 2013, seagrass, 2 m deep, colony fragment, up to 2.5 cm, on *Thalassia testudinum*. Stn. 2, May 2013, seagrass, 2.5 m deep, colony fragment, fertile, on *Thalassia testudinum*. Stn. 1, May 2013, seagrass, 2m deep, colony fragment, fertile, on *Thalassia testudinum*. Stn. 3, May 2013, seagrass, 2m deep, few polyps, stolonal, sterile, on *Thalassia testudinum*. Stn. 5, May 2013, seagrass, 2 m deep, colony fragment with one polyp, sterile, on *Thalassia testudinum*. Stn. 1, May 2013, seagrass, 2 m deep, colony fragment, sterile, on *Thalassia testudinum*. Stn. 1, May 2013, seagrass, 2 m deep, colony fragment, sterile, on *Thalassia testudinum*. Stn. 1, May 2013, seagrass, 2 m deep, colony fragment with two stolonal polyps, sterile, on *Thalassia testudinum*.

**Description.** Colonies of two types, stolonal and branched. Branched colony, monosiphonic, irregularly branched, whitish to brown in color. Annulations in some colonies can be found along the main stem and pedicels. Pedicels with 4 to 6 basal annulations, 134-2005  $\mu\text{m}$  in length and 55-331  $\mu\text{m}$  in wide. Hydranth total length 367-955  $\mu\text{m}$ , hydranth body length 221-687  $\mu\text{m}$ , hydranth diameter 212-513  $\mu\text{m}$ , Hypostome large and trumpet-shaped, 132-314  $\mu\text{m}$  length. Approximately 22–25 tentacles, in one whorl below hypostome. Abundant zooxanthellae in tentacles, hydranth body, hypostome and coenosarc. Spiny aspect of tentacles due to nematocyst distribution. Some polyps with at least five female gonophores, with simple spadix, in one whorl in the middle of the hydranth body.

**Cnidome (length x width).** Nematocysts of one category, heterotrichous microbasic euryteles, bean shaped, of two sizes--: small (5.8-8.8 x 2.4-4.7  $\mu\text{m}$ ), on tentacles, hydranth body, coenosarc, and scarcely on hypostome; large not discharged (12.2-17 x 4.2-9  $\mu\text{m}$ ), discharged (10-15.3 x 4.2-8.4  $\mu\text{m}$ , shaft 12.5-13  $\mu\text{m}$ ) in length, on hydranth body, hypostome and coenosarc.

**Remarks.** New record. *Eudendrium moulouyensis* is the only species of the genus *Eudendrium* that presents zooxanthellae (Marques et al 2000), a characteristic shared with the genus *Myrionema*, both belonging to the Eudendriidae family. However, differently from *Myrionema*, *E. moulouyensis* presents only a whorl of tentacles and its cnidome is composed of large heterotrichous microbasic euryteles, while *Myrionema* presents two or more whorl of tentacles and large macrobasic euryteles (De Vito et al., 2008).

**Great Caribbean records.** No Caribbean records.

**Geographical distribution.** Western and central Mediterranean Sea (Marques et al., 2000; Peña cantero and García Carrascosa, 2002; De Vito et al., 2008).

### ***Eudendrium bermudense* Calder, 1988**

(Fig 14. A-D)

**Material examined.** Stn. 8, March-2013, coral reef, 10 m deep. colony fragment with four polyps, up to 2 cm high, sterile. Stn. 8, March-2013, coral reef, 10 m deep, colony fragment with two polyps, up to 2.2 cm high, sterile. Stn. 11, March-2013, coral reef, 10 m deep, stolonal colony with 6 polyps, up to 4 mm, sterile.

**Description.** Stolonal or erect colony, alternately branched up to second order. Annulations: 3-4 at the base of pedicels, and occasional groups of 3-4 irregularly placed along the hydrocaulus. Diameter of pedicels 82-221  $\mu\text{m}$ . Hydranth total length 636-982  $\mu\text{m}$ , hydranth body length 178-674  $\mu\text{m}$ , hypostome knob-shaped to flared 82-307  $\mu\text{m}$  in length. Groove at the base of hydranth. Tentacles solid and filiform, in one whorl below hypostome, 20 to 24 in number.

**Cnidome.** Small heterotrichous microbasic euryteles (7.2-9.5 x 3.1-4.7  $\mu\text{m}$ ) on tentacles, hydranth body and coenosarc, and macrobasic euryteles (26-33 x 11.2-15  $\mu\text{m}$ ), abundant on hypostome, hydranth base, scattered on hydranth body and coenosarc.

**Remarks.** According to WoRMS, there are 9 accepted species of genus *Eudendrium* that possess large macrobasic euryteles in their cnidoma: *E. bermudense* Calder, 1988; *E. album* Nutting, 1898, *E. ritchiei* Millard, 1975; *E. infundibuliforme*, Kirkpatrick, 1890; *E. glomeratum* Picard, 1951; *E. simplex* Pieper, 1884; *E. aylingae* Watson, 1985; *E. cnidoferum* Stechow, 1919 and *E. currumbense* Watson, 1985. Of those, only *E. bermudense* and *E. album* have been reported for the Caribbean (Fraser, 1944; Calder, 1988, 1991 a, b, 2005; Calder and Cairns, 2009).

Unlike our material, *E. album* have the basal third of the tentacles almost free of nematocysts, and in the most distal region, the nematocysts are arranged in rings (Puce et al., 2005; Schuchert, 2008 b). *E. cnidoferum* and *E. glomeratum* have the macrobasic euryteles in buttons and are absent on hypostome (Schuchert, 2008 b), in our material; macrobasic euryteles are abundant on hydranth base and hypostome. *E. ritchiei* has perisarc strongly annulated and is endemic to South Africa, (Millard, 1795), unlike our material. The pedicels in *E. infundibuliforme* expand distally unlike the samples under study. *E. aylingae* has only been reported for Australia and Indonesia, and is much smaller than *E. bermudense*, (Calder, 1988).

According to Watson (1990), macrobasics in *E. currumbense* are just a few, scattered on hydranth and hypostome, unlike the study samples that have abundant macrobasic in hypostome and hydranth base. *E. infundibuliforme* is considered endemic to Australia, (Marques et al., 2000), and has two kinds of macrobasic euryteles (Watson, 1990), our material has only one kind of them. *E. simplex* is a Mediterranean species, predominantly or perhaps exclusively on *Posidonia oceanica*, (Schuchert, 2008 b), which is not present in the study area. For those reasons, and due to its distribution close to the study area, it was decided to identify our material as *Eudendrium bermudense*. However, samples with gonophores are needed for more precise identification.

**Great Caribbean records.** Bermudas (Calder, 1988).

**Geographical distribution.** Only Caribbean records.

***Eudendrium album* Nutting, 1898**

(Fig 15. A-C)

**Synonymy.** Complete synonymy in Schuchert (2008 b).

**Material examined.** Stn. 12, March-2013, coral reef, 10 m deep, fragment of delicate stolonal colony, a few polyps, up to 1.8 cm high, sterile, on alga. Stn. 12, March-2013, coral reef, 10 m deep, two polyps with pedicels, up to 2.5 mm high, without gonophores. Stn. 8, March-2013, coral reef, 10 m deep, colony fragment, branched, up to 1 cm high, sterile. Stn., 13, March-2013, coral reef, 10 m deep, colony fragment with two polyps, small and fragile, sterile.

**Description.** Erect or stolonal colony, small, monosiphonic, irregularly branched. Perisarc smooth with 3-4 annulations at the base and groups of 2-3 annulations along the pedicel, diameter of pedicels 57-172  $\mu\text{m}$ . Hydranth cup shaped, with 286-421  $\mu\text{m}$  in total length, hydranth body length 167-271  $\mu\text{m}$ . Hypostome rounded, 82- 157  $\mu\text{m}$ , approximately 17-20 tentacles, in one whorl below hypostome, basal third of tentacles nearly free of nematocysts.

**Cnidome.** Small heterotrichous microbasic euryteles (5-9 x 2.3-4.6  $\mu\text{m}$ ) on tentacles, hydranth body and coenosarc, and macrobasic euryteles (17-25 x 6.2–10  $\mu\text{m}$ ), on hypostome, hydranth body and coenosarc.

**Remarks.** Of the nine species of eudendrium with macrobasic euryteles, only *E. bermudense* and *E. album* have been reported for the Caribbean (Fraser, 1944; Calder ,1988, 1991 a, b, 2005; Calder and Cairns, 2009). The size of the macrobasics in our material is like that reported by Schuchert (2008 b, 2012) for *E. album*. According to Schuchert (2012). The combination of small, monosiphonic colonies and the presence of large macrobasic euryteles is diagnostic character for *E. album*.

**Great Caribbean records.** EE.UU (Fraser, 1944); Panamá (Calder, 2005).

**Geographical distribution.** Mediterranean to North Atlantic (Schuchert, 2012). See specific records in Schuchert (2008 b).

***Eudendrium* sp1.**

(Fig 16. A-D)

**Material examined.** Stn. 2, May 2013, seagrass, 2 m deep, colony fragment with three polyps, up to 4.4 mm high, on *Thalassia testudinum*, sterile.

**Description.** Erect colony alternately branched. Annulations: groups of 4-5 along the hydrocaulus, 3-4 at the base of pedicels., diameter of pedicel 72-77  $\mu\text{m}$ , 85  $\mu\text{m}$  of hydrocaulus. Hydranth total length 1.1 mm, hydranth body length 879  $\mu\text{m}$ , hypostome trumpet shaped 198  $\mu\text{m}$  in length. Nearly 22 Tentacles solid and filiform, in one whorl. Spiny aspect of tentacles due to nematocysts distribution.

**Cnidome (length x width).** Small heterotrichous microbasic euryteles (4.5-6.1 x 2.7-3.7  $\mu\text{m}$ ) abundant on tentacles, hydranth body and scattered in coenosarc; ring of large microbasic euryteles in the middle of the hydranth body (18-23.4 x 13.1-14.8  $\mu\text{m}$ ).

**Remarks.** Unlike the other samples of *Eudendrium* of this study, this species has a ring of large microbasic euryteles in the middle of the hydranth body, which also differs from the *Eudendrium* species with large microbasic euryteles, reported for the Caribbean. For those reason, it was decided to identify our material as *Eudendrium* sp. 1. Samples with gonophores are needed for precise identification.

### **Genus *Myrionema* Pictet, 1893**

#### ***Myrionema amboinense* Pictet, 1893**

(Fig 17. A-G)

**Synonymy.** *Eudendrium hargitti* Congdon, 1906: 27; figs. 5-11. *Myrionema hargitti*: Spracklin, 1982: 240; fig. 114 b

**Material examined.** Stn. 1, May 2013, seagrass, 2 m deep, two polyps, fertile, on *Thalassia testudinum*. Stn. 1, May 2013, seagrass, 2 m deep, one stolon with one single polyp, up to 1.6 cm, sterile, on *Thalassia testudinum*. Stn. 1, May 2013, seagrass, 2 m deep, stolonial colony up to 1.2 cm, sterile, on *Thalassia testudinum*. Stn. 5, May 2013, seagrass, 2 m deep, stolonial colony up to 1.5 cm, sterile, on *Thalassia testudinum*. Stn. 5, May 2013, seagrass, 2 m deep, stolonial colony up to 1.5 cm, sterile, on *Thalassia testudinum*. Stn. 4, May 2013, seagrass, 2,8

m deep, small and stolonal colony, few polyps, fertile, on *Thalassia testudinum*. Stn. 5, May 2013, seagrass, 2 m deep, sterile, on *Thalassia testudinum*. Stn. 1, May 2013, seagrass, 2 m deep, sterile, on *Thalassia testudinum*. Stn. 1, May 2013, seagrass, 2 m deep, fertile, on *Thalassia testudinum*. Stn. 1, May 2013, seagrass, 2 m deep, fertile, on *Thalassia testudinum*. Stn. 1, May 2013, seagrass, 2.5 m deep, three polyps with long pedicels, up to 1.5 cm, fertile, on *Thalassia testudinum*. Stn. 1, May 2013, seagrass, 2 m deep, fertile, on *Thalassia testudinum*. Stn. 1, May 2013, seagrass, 2.5 m deep, fertile, on *Thalassia testudinum*. Stn. 1, May 2013, seagrass, 2.5 m deep, stolonal colony, three polyps, sterile, on *Thalassia testudinum*.

**Description.** Colony of two types, stolonal and branched, monosiphonic, with creeping hydrorhiza, growing on *Thalassia testudinum*. Annulations at the base of hydrocaulus, branches and pedicels. Pedicel's length 1.7-2 mm, and 77-159 µm wide. Hydranths, with 265-1282 µm in total length, hydranth body elongate, 257-922 µm in length, hypostome, flared to knobbed, 82-449 µm in length, with as many as 30 or more filiform tentacles arranged in two or more closed whorls, around hypostome. Abundant zooxanthellae in tentacles, hydranth body, hypostome and coenosarc of hydrocaulus. Gonophores as fixed sporosacs, Male gonophores, in number of two, each one with three chambers, originating on hydranth body, proximal to tentacles. Immature female gonophores originating in a whorl, on hydranth body, with four eggs, supported by simple spadix. Spadix curving over eggs. Mature female gonophores with three eggs distributed along the pedicels.

**Cnidome (length x width).** Nematocysts small heterotrichous microbasic eurytele (7.5-11.2 x 2.5-4.9 µm) on tentacles, hydranth body and coenosarc, and large macrobasic eurytele (17.3-24.8 x 7.1-10.7 µm) on hypostome, gonophores and coenosarc of hydrocaulus. At the base of some hydrants, there is a large ring of macrobasic euryteles.

**Remarks.** Confirmation. Varela (2012) reported for the first time the genus *Myrionema* and the species *M. amboinense* for Cuban waters. According to Calder (1988), the genus *Myrionema* Pictet (1893) is apparently restricted to shallow-water habitats in the tropics and subtropics. According to WoRMS, there are two species in the genus, *M. amboinense* Pictet, 1893 and *M. hargitti* (Congdon, 1906), which Calder (1988) considers conspecifics, because the hydranth length and number of tentacles are variable characters not sufficient to differentiate the species.



**Great Caribbean records.** Belize (Spracklin, 1982, as *Myrionema hargitti*; Calder, 1991a, b); Colombia (Flórez González, 1983; Bandel and Wedler, 1987; as *M. hargitti*); Puerto Rico (Wedler and Larson, 1986, as *M. hargitti*); Cuba (without description) (Varela, 2012).

**Geographical distribution.** Eastern (Picard, 1958) and western Atlantic (Fraser, 1944); Indian Ocean, Millard, and Bouillon, 1973; Pacific Ocean (Pennycuik, 1959); Mediterranean, Marques et al., 2000.

### **Family Oceaniidae Eschscholtz, 1829**

#### **Genus *Corydendrium* Van Beneden, 1844**

#### ***Corydendrium parasiticum* (Linnaeus, 1767)**

(Fig 18. A-D)

**Synonymy.** *Sertularia parasitica* Linnaeus, 1767: 1315. *Soleniopsis dendriformis* Ritchie, 1907. Complete synonyms in Schuchert (2004).

**Material examined.** Stn. 10, March-2013, coral reef, 10 m deep, five stolonal polyps, and two branched colony fragments with two polyps each, up to 2.5 mm, sterile. Stn. 11, March-2013, coral reef, 10 m deep, colony fragment with one polyp, up to 1,5 mm. Stn. 11, March-2013, coral reef, 10 m deep, colony polysiphonic up to 2 cm, sterile, on octocoral.

**Description.** Colony stolonal or erect polysiphonic, up to 2 cm high arising from a creeping hydrorhiza. Irregular branching in one or more planes. In its basal portion the branches are attached to the main axis and gradually they separate towards the distal portion. Perisarc moderately thick, becoming thin in the distal parts of the branches. Pedicel length 1.2 mm with 81-261 µm in diameter in basal parts to 385-473 µm in distal parts. Hydranth spindle-shaped, constricted basally below the distal end of perisarc tube. Hydranth total length 1.5 mm. Hypostome elongated, 255 µm in length in relaxed polyps. Approximately 18 filiform tentacles distributed irregularly along the hydranth body.

**Cnidome (length x width).** Desmonemes (3.4-4.3 x 2-3.3 µm) and heterotrichous microbasic euryteles (8.1-8.7 x 3.9-4.8 µm), in hydranth body, tentacles and coenosarc of hydrocaule.



**Remarks.** Confirmation record. Among the six species of the genus *Corydendrium* accepted in WoRMS, *C. parasiticum* is the only reported for Cuba and for the Caribbean.

**Great Caribbean records.** Belize (Spracklin 1982); Bermudas (Calder 1988); Colombia (Wedler 1975, Flórez Gonzáles 1983); Cuba (without description) (Varela, 2005); Martinique (Galea, 2013); Panamá (Calder, 2005); Puerto Rico (Wedler and Larson 1986).

**Geographical distribution.** Western (Wedler, 1975) and eastern Atlantic (Ritchie, 1908); Indian Ocean (Millard, 1975); western (Leloup, 1937) and eastern Pacific (Fraser, 1938).

### **Genus *Rhizogeton* Agassis, 1862**

#### ***Rhizogeton sterreri* (Calder, 1988)**

(Fig 19. A-D)

**Synonymy.** *Rhizodendrium sterreri* Calder, 1988.

**Material examined.** Stn. 14, March-2013, coral reef, 10 m deep, colony up to 1.7 mm, with two polyps, sterile.

**Description.** Stolonal and sessile hydranth, on creeping hydrorhiza. Perisarc thin, covering the base of the hydranth. Pedicel length 531  $\mu\text{m}$ . Hydranth cylindrical, with 1.1 mm in total length hydranth body length 945  $\mu\text{m}$  and 249  $\mu\text{m}$  wide. Hypostome dome-shape, 176  $\mu\text{m}$  in length. Approximately 23 filiform tentacles, whose length decreasing from distal to proximal part of hydranth body; distal tentacles also more robust than proximal ones.

**Cnidome (length x width).** Nematocysts heterotrichous microbasic euryteles (5.4-7.1 x 2.1-2.8  $\mu\text{m}$ ) and desmonemes (4.2-5 x 2.6-3.2  $\mu\text{m}$ ) distributed all over tentacles, hydranth body and hydrocaulus.

**Great Caribbean records.** Bermudas (Calder, 1988) as *Rhizodendrium sterreri*; Guadeloupe and Martinique (Galea, 2008); Mexico and Yucatan (Calder and Cairns, 2009).

**Geographical distribution.** Brazil, Kelmo and St Isabel (1998) as *Rhizodendrium sterreri* Calder, 1988.

***Rhizogeton conicum* Schuchert 1996**

(Fig 20. A-D)

**Material examined.** Stn. 1, May 2013, seagrass, 2 m deep, colony fragment with two complete polyps and three pedicels, up to 2.3 mm, sterile. Stn. 1, May 2013, seagrass, 2 m deep, colony fragment with two complete polyps and four pedicels, sterile. Stn. 8, March-2013, coral reef, 10 m deep, one polyp, up to 2.2 mm, sterile. Stn. 14, March-2013, coral reef, 10 m deep, colony fragment with two polyps, sterile. Stn. 14, March-2013, coral reef, 10 m deep, two polyps, up to 2 mm, sterile.

**Description.** Stolonal colony, with creeping hydrorhiza covered with thin perisarc. Perisarc smooth or wrinkled. Pedicels increasing in diameter from the base (106-144  $\mu\text{m}$ ) to distal end (180-286  $\mu\text{m}$ ), into which the polyp can withdraw completely, or partially. Hydranth cylindrical to spindle shaped, with 552-1316  $\mu\text{m}$  in total length, and 212-318  $\mu\text{m}$  wide, hydranth body length 1.2 mm, with approximately 19 filiform tentacles organized in three to four 4 whorls in the distal part of the hydranth, below hypostome. Hypostome short, conical or dome-shape, 100  $\mu\text{m}$  length.

**Cnidome (length x width).** Heterotrichous microbasic euryteles (8.1-9 x 3.6-4  $\mu\text{m}$ ) Heterotrichous microbasic euryteles more bean shape than the others (10.8-11.7 x 4-5  $\mu\text{m}$ ) and desmonemes (4.9-6 x 3-6.8  $\mu\text{m}$ ) distributed all over tentacles, hydranth body and coenosarc of hydrocaulus.

**Remarks.** As Schuchert (1996) comments, *Rhizogeton conicum* is the unique species within this genus having a conical perisarc covering the pedicel into which the polyp can withdraw completely, or at least partially, in older polyps. The polyps of our material are not totally relaxed, that is why the hydranth has a spindle shape and not cylindrical, and tentacles do not appear much scattered as in the original description.

**Great Caribbean records.** No Caribbean records.

**Geographical distribution.** New Zealand. Known only from Wellington's south coast.

**Genus *Turritopsis* McCrady, 1857**

***Turritopsis nutricula* McCrady, 1857**

(Fig 21. A-D)

**Synonymy.** *Corydendrium nutricula* (McCrady, 1857) (unaccepted combination). *Modeeria multitentaculata* Fewkes, 1881 (synonym).

**Material examined.** Stn. 8, March 2013, coral reef, 10 m deep, branched colony with five polyps, up to 6 mm, fertile. Stn. 8, March 2013, coral reef, 10 m deep, branched colony, sterile. Stn. 11, March 2013, coral reef, 10 m deep, branched colony, sterile.

**Description.** Colony erect, irregularly branched, monosiphonic. Branches adnated to hydrocaulus at their origin and becoming free in distal part. Firm perisarc, with two layers, without annulations, increasing in diameter from the base (143  $\mu\text{m}$ ) to the distal end (223  $\mu\text{m}$ ) below hydranth base. Hydranth terminal, naked, elongated, fusiform, with approximately 15 filiform tentacles scattered on the body. Hypostome elongated and conical. Gonophores as medusa buds, on pedicels.

**Cnidome (length x width).** Nematocysts heterotrichous microbasic euryteles (5.1-6.3 x 2.5-2.7  $\mu\text{m}$ ) and desmonemes (3.1-3.5 x 2-2.3  $\mu\text{m}$ ) on tentacles, hydranth body and coenosarc of hydrocaulus.

**Remarks.** According to recent morfological and molecular studies (Miglietta et al., 2007; Miglietta, 2016; LI et al., 2018) the *Turritopsis* species, form three exclusive lineages: *T. nutricula* McCrady, 1857 in Western Atlantic, *T. dohrnii* (Weismann, 1883) in Mediterranean Sea, and *T. rubra* (Farquhar, 1895) in Pacific Ocean.

**Great Caribbean records.** Panamá, Curaçao, and Virgin Islands (Stechow, 1919); Cuba (without description), (Varela et al., 2010 Castellanos-Iglesias et al., 2018); EE.UU., (Deveey, 1954); Belize, (Spracklin, 1982); Puerto Rico, (Wedler and Larson, 1986); Bermudas, (Calder, 1988); Guadeloupe, (Galea, 2008); Martinique, (Galea, 2013).

**Geographical distribution.** Gulf coast of Florida and elsewhere Western Atlantic, Calder (2019).

#### 4. Conclusions

The present study reports 20 species of athecate hydroids, for the Cuban marine platform waters, constituting the first study directed to taxonomy of “Anthoathecata” group in the country. Of the 20 species identified eight species are confirmations, and 11 species are new additions to the Cuban hydroid's fauna: *Cladocoryne floccosa*, *Slabberia strangulata*, *Zanclaea alba*, *Millardiana longitentaculata*, *Pachycordyle napolitana*, *Eudendrium bermudense*, *Eudendrium album*, *Eudendrium klausi*, *Eudendrium moulouyensis*, *Rhizogeton sterreri*, and *Rhizogeton conicum*. Of them, two constitutes new records for the Caribbean waters: *E. moulouyensis*, species reported as endemic to the Mediterranean, Bouillon et al. (2004), De Vito et al. (2008), and *Rhizogeton conicum*, reported only from Wellington's south coast in New Zealand. The presence of symbiotic zooxanthellae is a diagnostic character of this species within the genus *Eudendrium*. The identification of the materials in this study was based mainly on the characteristics of the cnidome, the gonophores when they were present and other morphology data commonly used in taxonomy.

The 24 species of athecate hydroids reported in the literature before this study for Cuban waters, come from collections made in the western and central region of the country, in the north and south, including Isla de la Juventud. Except for *Pennaria disticha*, whose description as *P. symmetrica* Clarke, 1879, was based on material collected in Bahia Honda, Cuba, the other confirmations are described for the first time for Cuba in this study. Of those previous records, 15 species were recorded in the north of Havana, 1 in Artemisa, 4 in the south of Matanzas, 3 in Pinar del Rio, 1 on the Isla de la Juventud, 1 in the archipelago Canarreos and 1 in the Gulf of Batabanó.

The best represented habitat was coral reefs with 14 species. In seagrass meadows were found 7 species. The *Eudendrium* genus, present in both habitats, was better represented in the coral reef habitat, with 5 species, versus seagrass meadows with 2 species. Possibly due to the greater diversity of substrates present in the first habitat. Only one species was shared by the two habitats: *Rhizogeton conicum*, which constitute a new record for Cuba.

As a result of this study, the checklist of the athecate hydroids increased to 35 species, for 5 of those, reproductive structures are described also for the first time for the country. The

largest number of records and the most diverse area corresponds to northern Havana, but it is also the most studied area. There is a great probability of continuing to find even new records of hydroids in Cuba, there are many areas of the marine platform where this group has not been studied, so a recommendation, is encourage to continue with the taxonomic studies of hydroids in Cuba, that include the eastern region of the country, and enriching them with systematic studies which would contribute to a better identification of the species and a better knowledge of the diversity of this group in the country, and in the Caribbean region.

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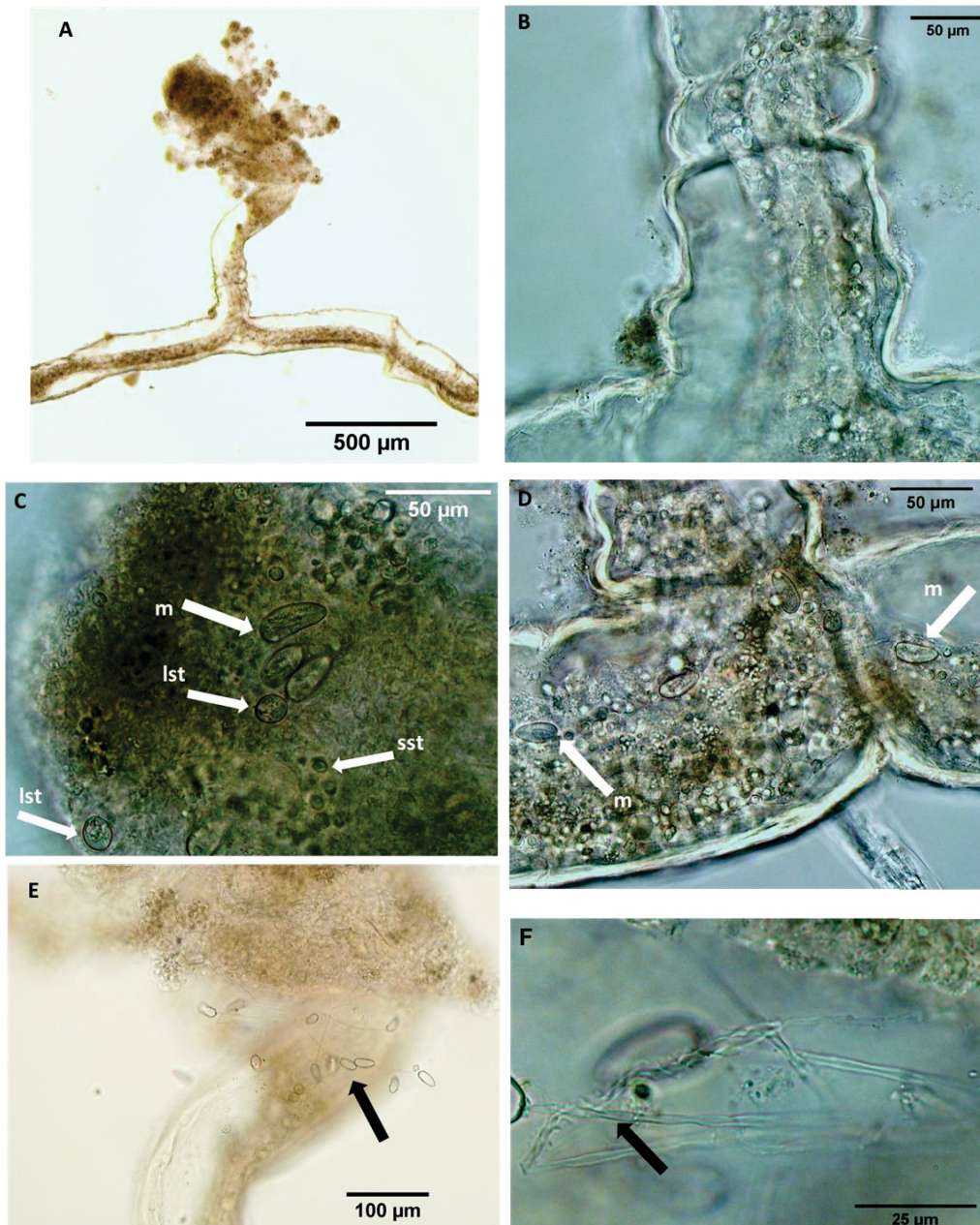
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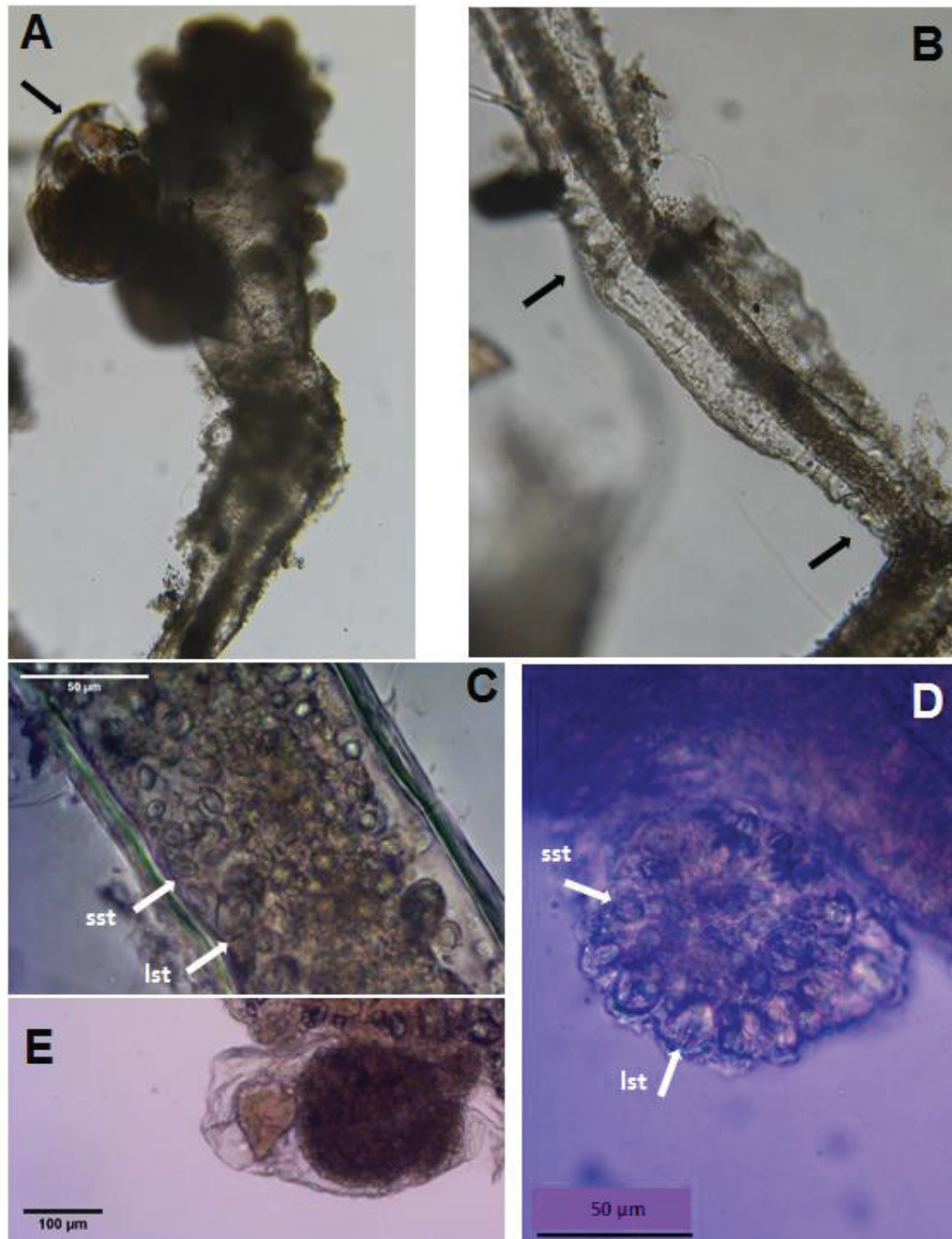
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## Figures

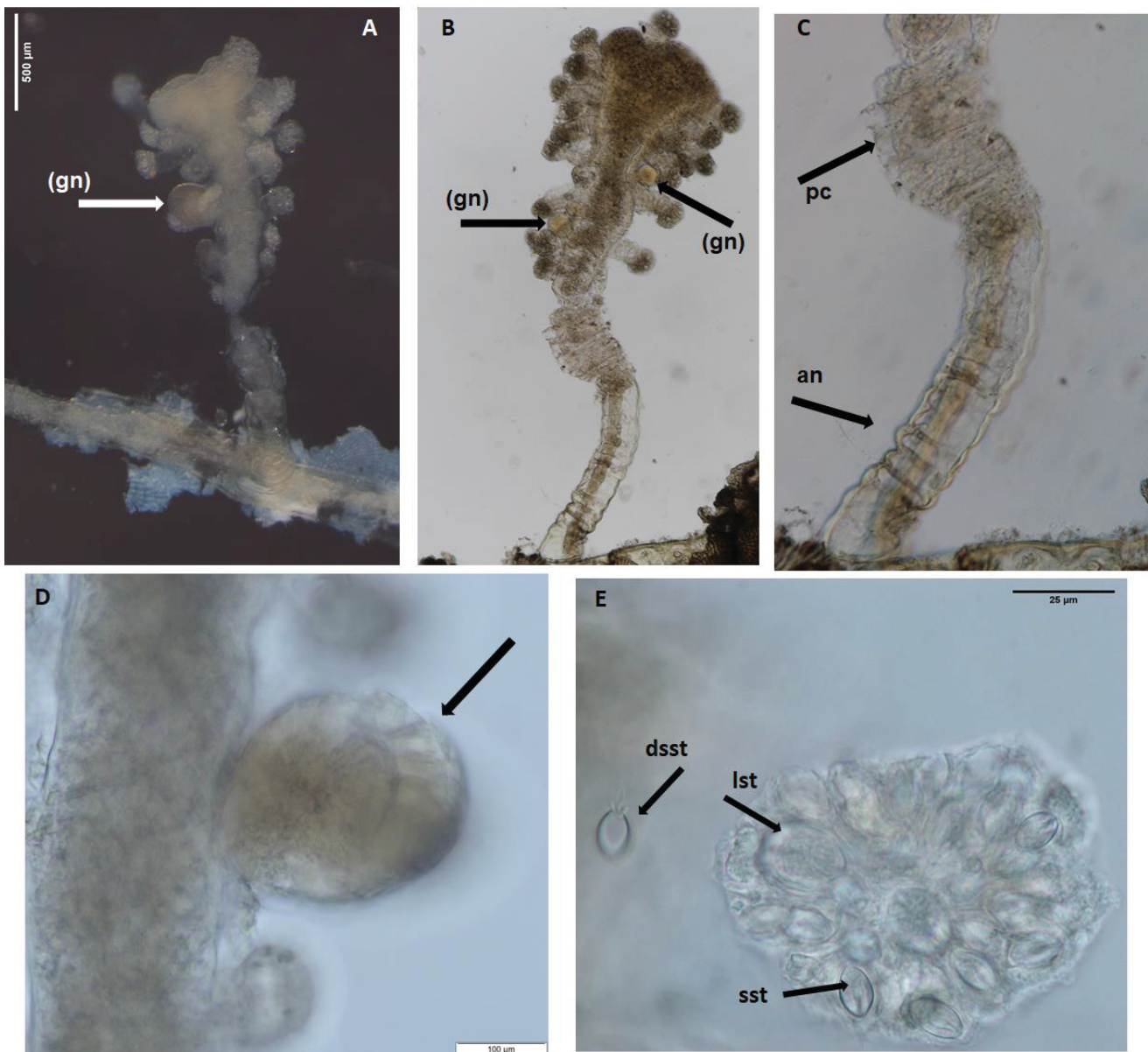


**Figure 2.** *Cladocoryne floccosa* A- stolonal colony fragment with one polyp, B- detail of annulations in pedicel, C- nematocyst pouche between the base of the oral tentacles, D- nematocysts in coenosarc, E- discharged macrobasic euryteles between the proximal whorl of aboral tentacles (arrow), F- detail of shaft in discharged macrobasic euryteles (arrow). **sst** = small stenotele, **lst** = large stenotele, **m** = macrobasic euryteles.

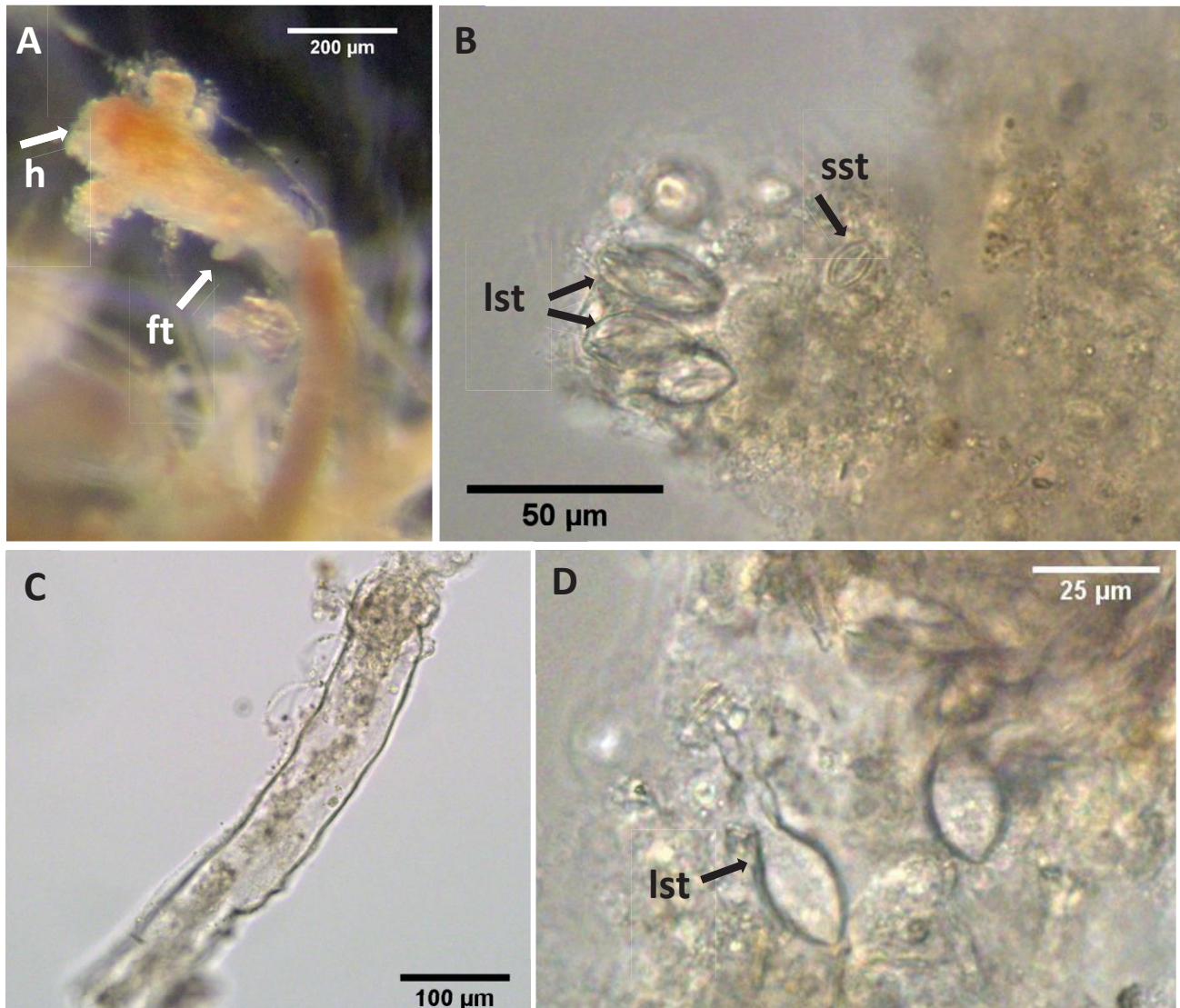


**Figure 3.** *Coryne pusilla* A- Hydranth with gonophore (sporosac) (arrow), B- Annulations in the medial and basal parts of pedicel. C- Stenoteles in coenosarc, and D- in capitule tentacles, E- Detail of the gonophore. **sst** = Small stenoteles, **lst** = large stenoteles

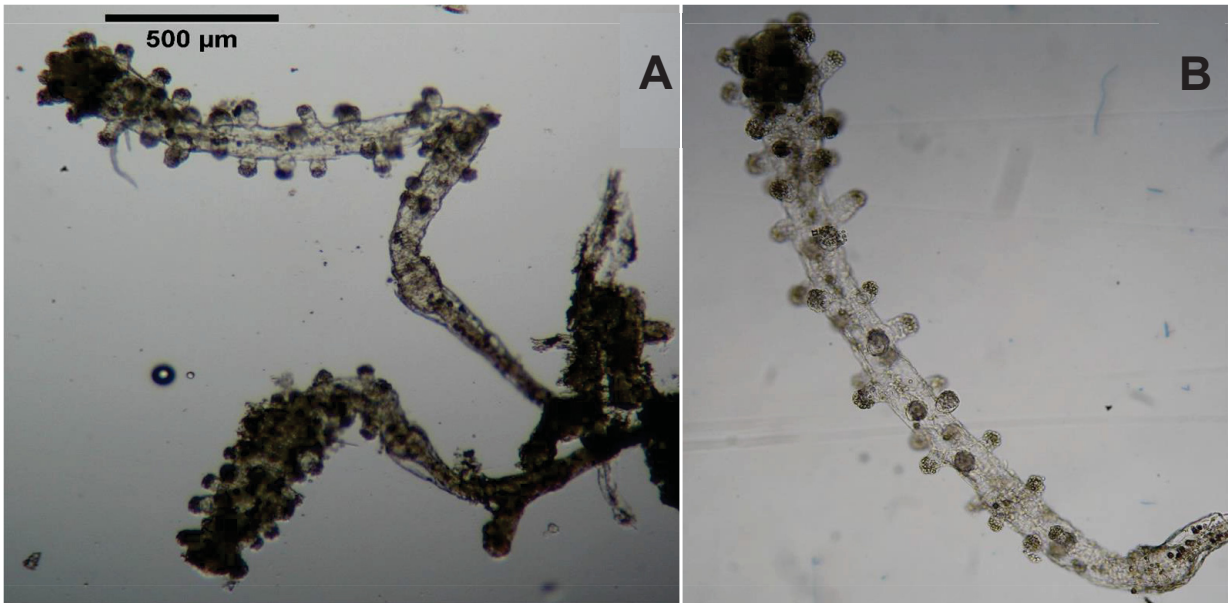




**Figure 4.** *Coryne* sp1. A and B- Colony fragment with one polyp, hydranth with gonophores, C- Detail of annulations and perisarc collar. D- Detail of gonophore, E- stenoteles in capitate tentacle knob, and small stenotele discharged. **gn** = gonophore, **an** = annulations, **pc** = perisarc collar, **sst** = small stenoteles, **lst** = large stenotele, **dsst** small stenotele discharged.

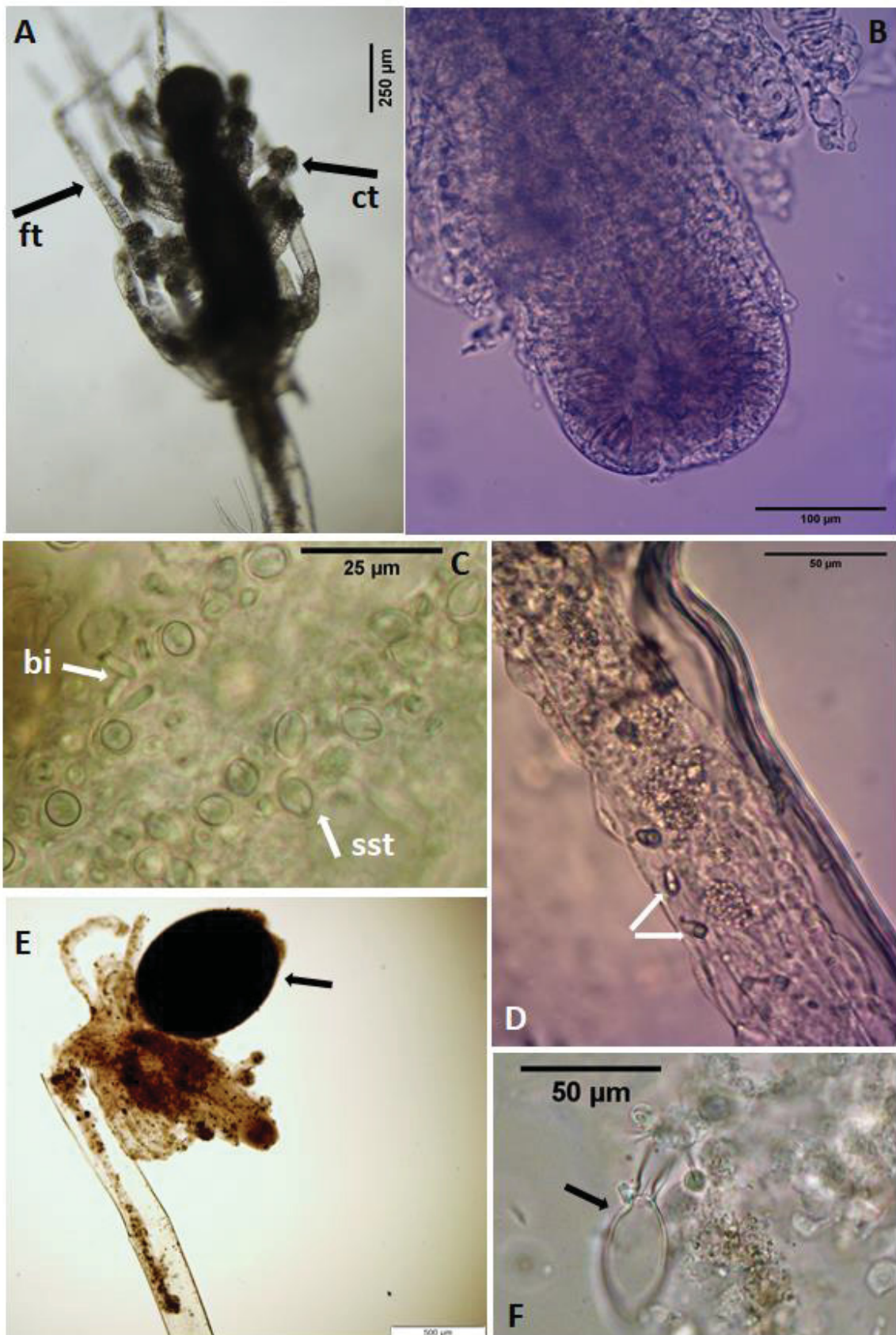


**Figure 5.** *Slabberia strangulata* A- hydranth with pedicel, B- hydranth with stenoteles in capitate tentacles, C- pedicel, D- discharged large stenotele (arrow). **h** = hypostome, **ct** = capitate tentacles, **ft** = filiform tentacles, **lst** = large stenotele, **sst** = small stenotele.

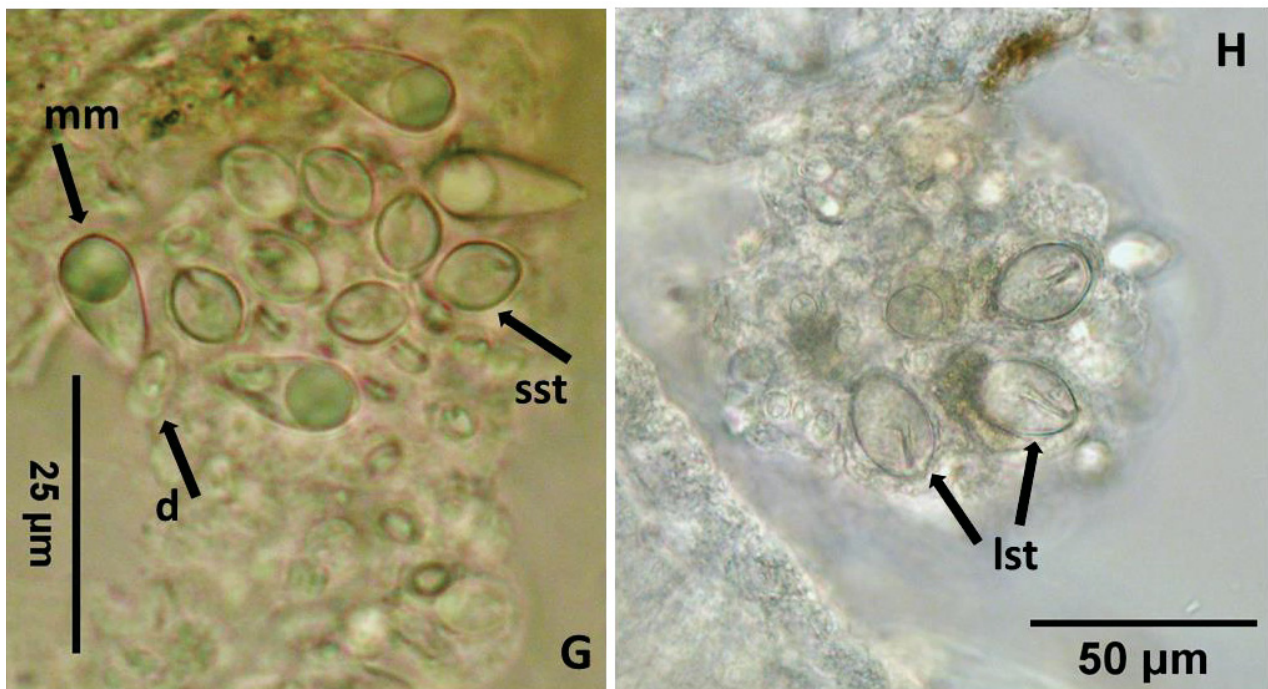


**Figure 6.** *Zanclea alba* A- Colony fragment with two stolonal polyps, B- hydranth.

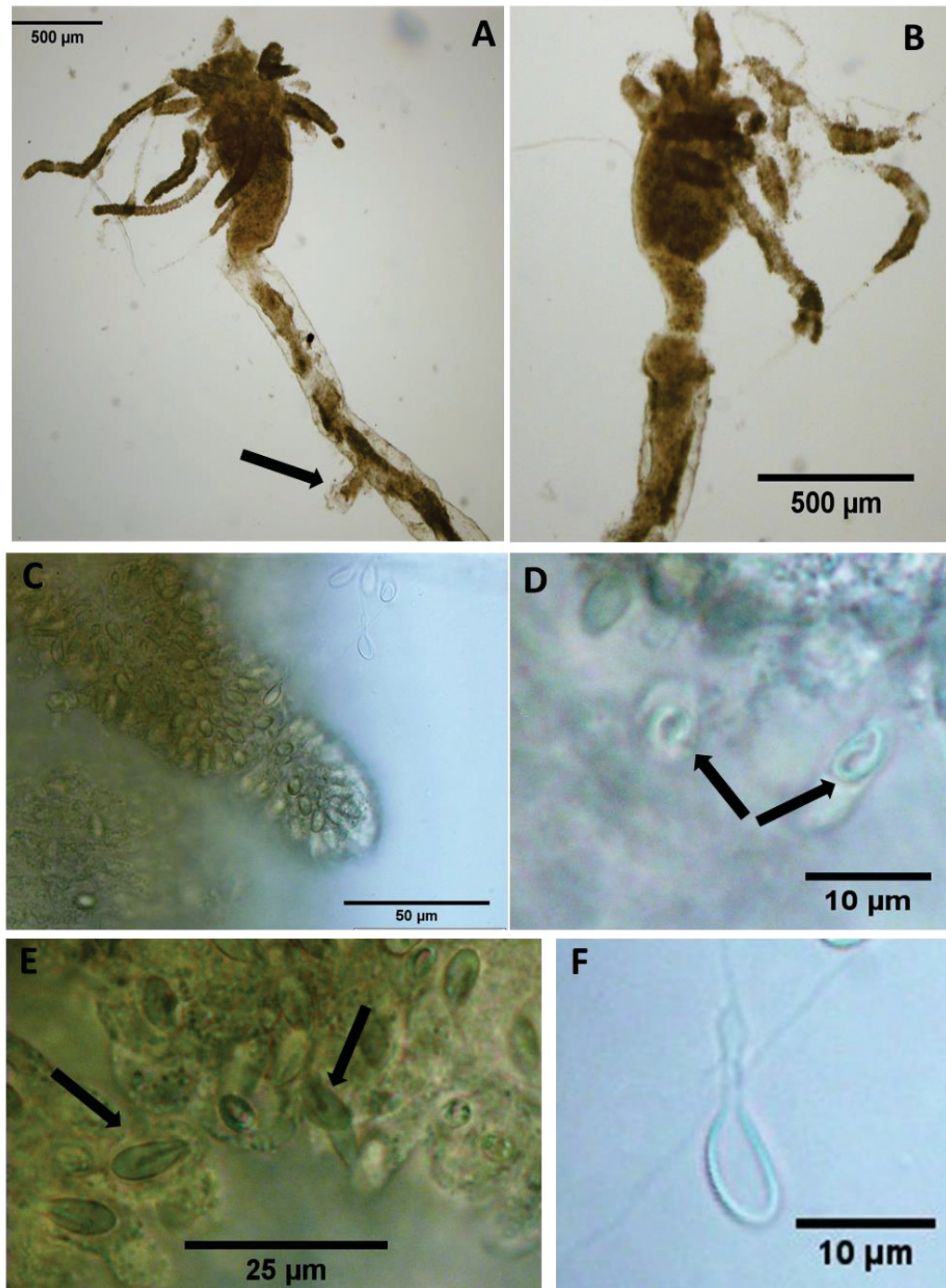




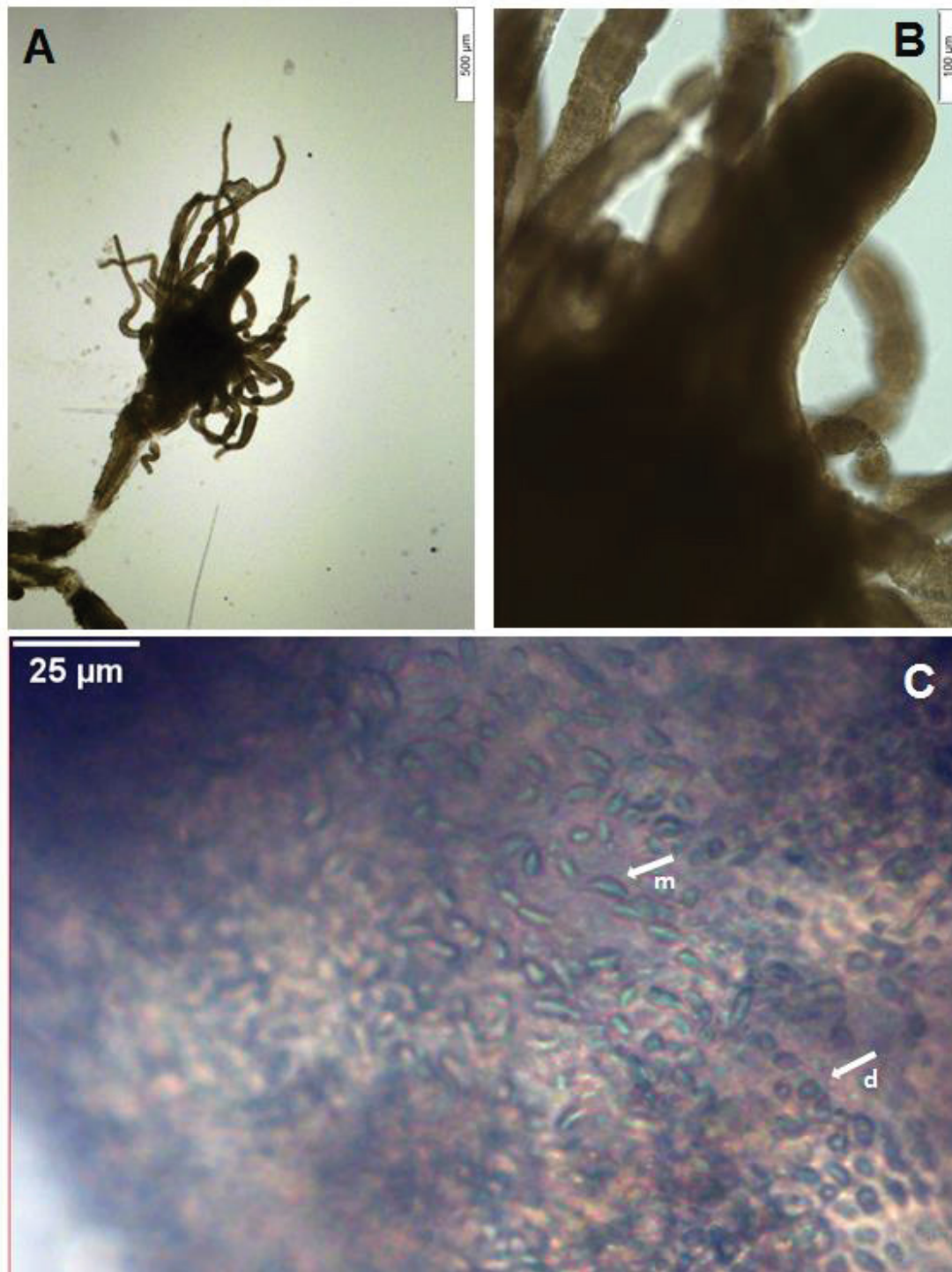




**Figure 7.** *Pennaria disticha* A- Hydrant with capitate and filiform tentacles, B- Detail of hypostome, C- Nematocysts, D- Nematocysts: microbasic b mastigophore with inclusion in the coenosarc of hydrocaulus (arrows), E- Hydrant with one gonophore (arrow), F.- discharged stenotele, G- nematocysts, H- nematocysts in capitate tentacles. **ct** = capitate tentacles, **ft** = filiform tentacles, **bi** = basitrichous isorhizas? **sst** = small stenotele, **mst** = medium stenotele, **lst** = large stenotele, **mstd** = medium stenotele discharged, **d** = desmoneme, **mm** = microbasic mastigophore with basal sphaerical inclusion.

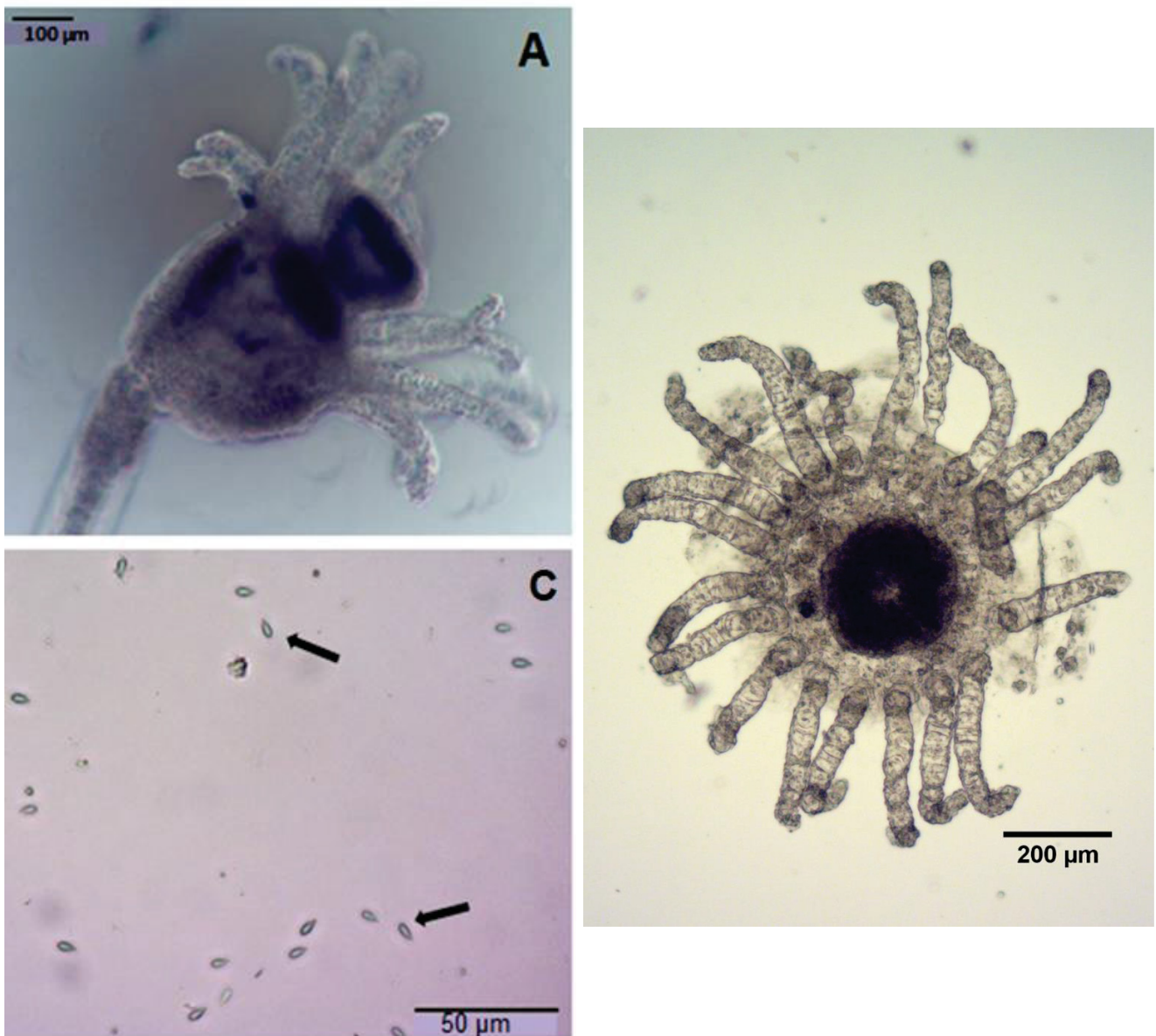


**Figure 8.** *Pachycordyle napolitana* A hydranth, with possible gonophore on pedicel (arrow), B hydranth, C- detail of tentacle, D- desmonemes (arrows), E- small microbasic euryteles undischarged (arrows), F- microbasic euryteles discharged.

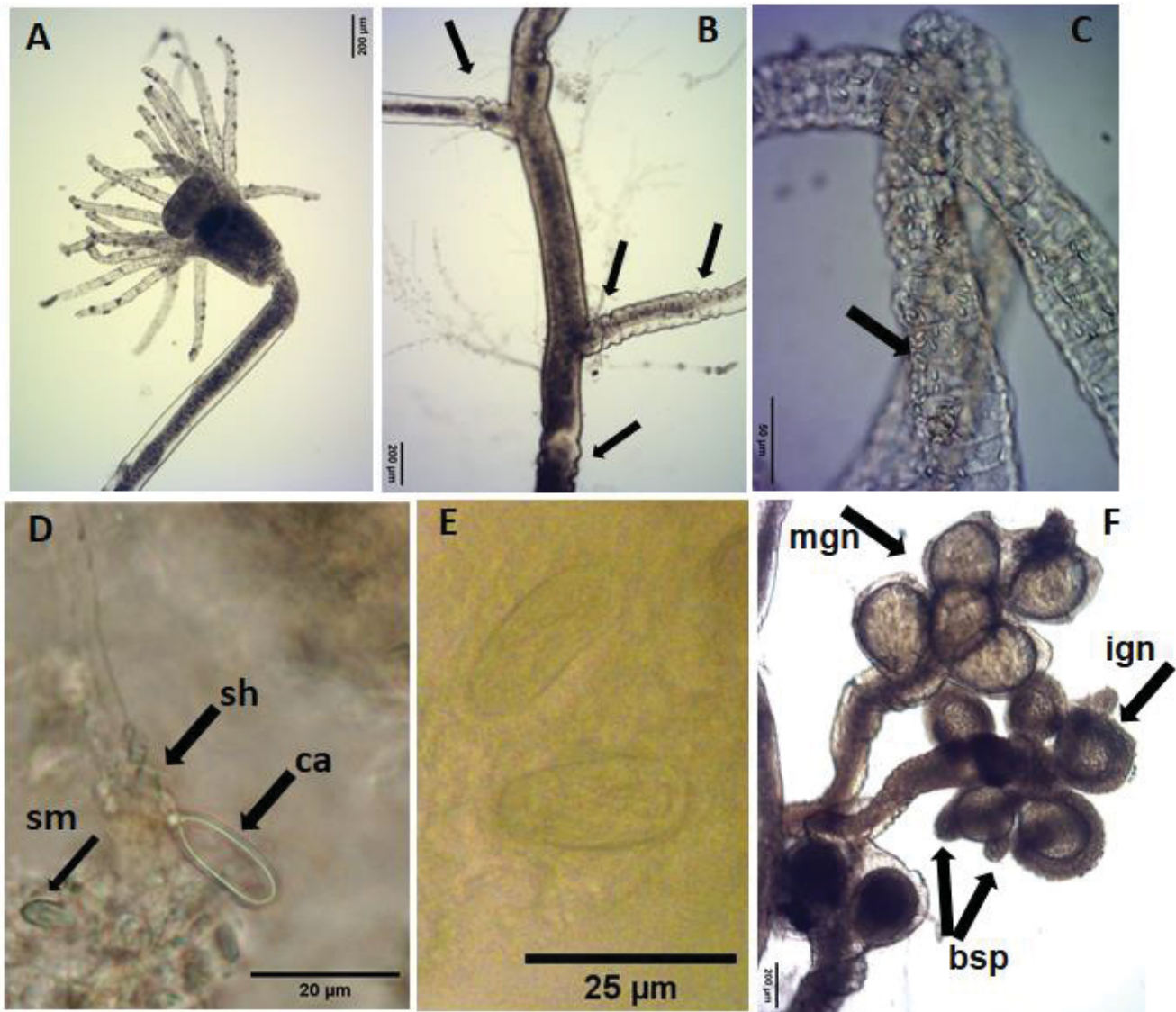


**Figure 9.** *Millardiana longitentaculata* A- Hydranth, B- Detail of hypostome, C- nematocyst in hydranth body. **m** = small microbasic euriteles, **d** = desmonemes.

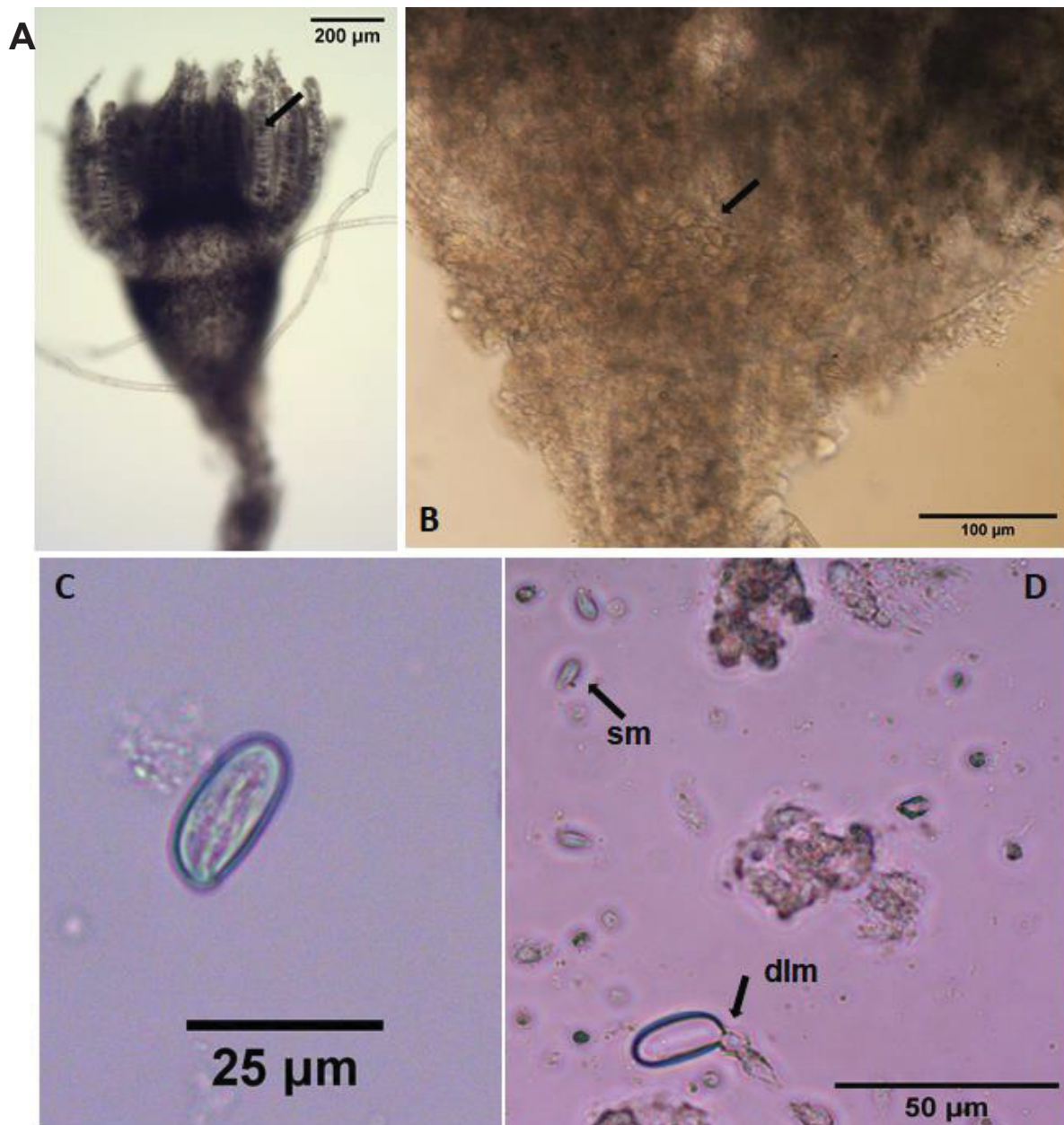




**Figure 10.** *Eudendrium capillare* A- Hydranth, B- hydranth top view, C- Small heterotrichous microbasic euryteles (arrows).

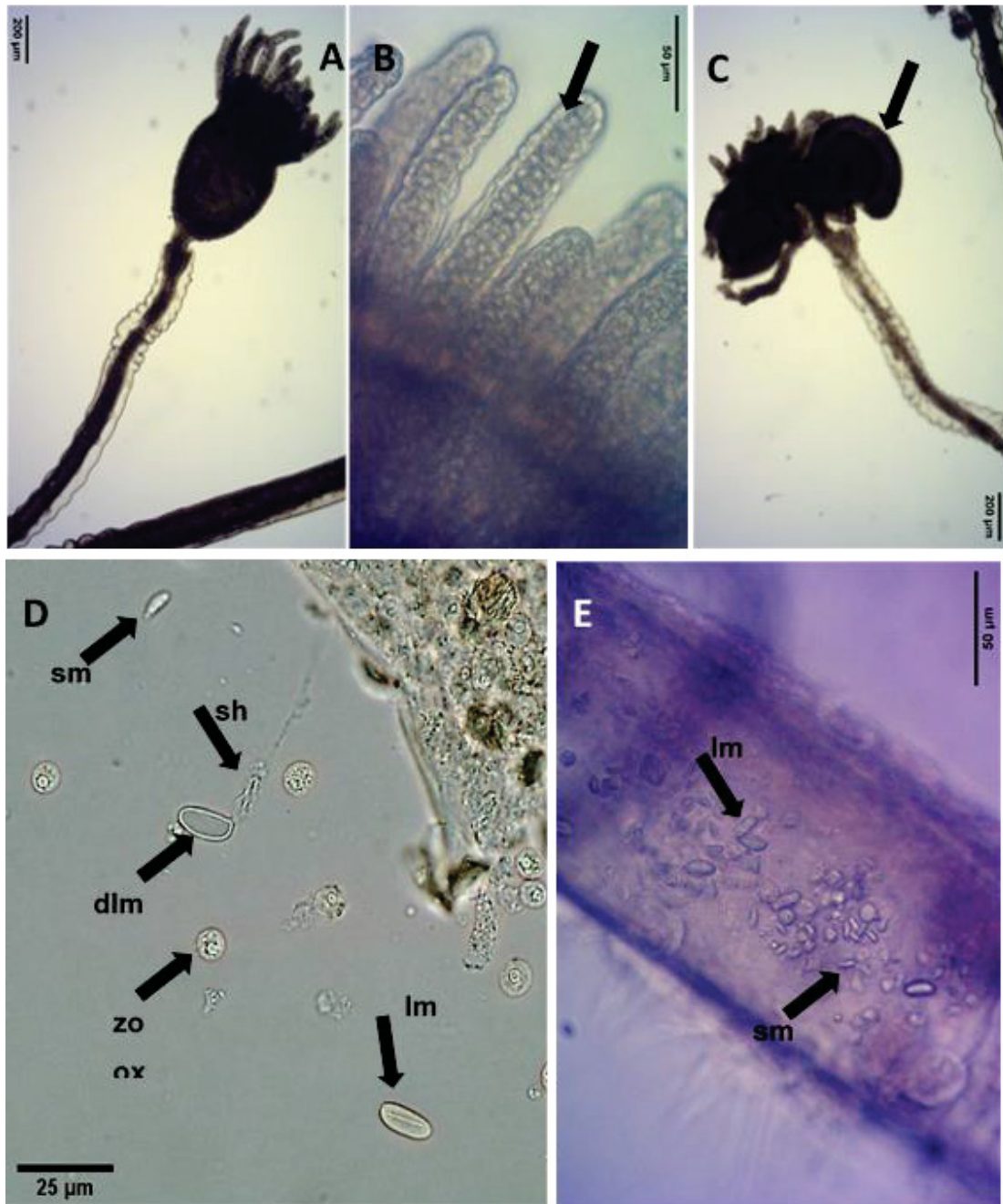


**Figure 11.** *Eudendrium carneum*. A- hydranth, B- Annulations in pedicels (arrows), C- small microbasic euriteles in tentacles (arrow), D- discharged heterotrichous anisorhiza (arrows), E- nematocysts anisorhizas undischarged, D- female gonophores. **sh** = shaft, **ca** = capsule, **sm** = small microbasic eurytele, **mgn** = mature gonophores, **ign** = immature gonophores, **bsp** = bifid spadix.

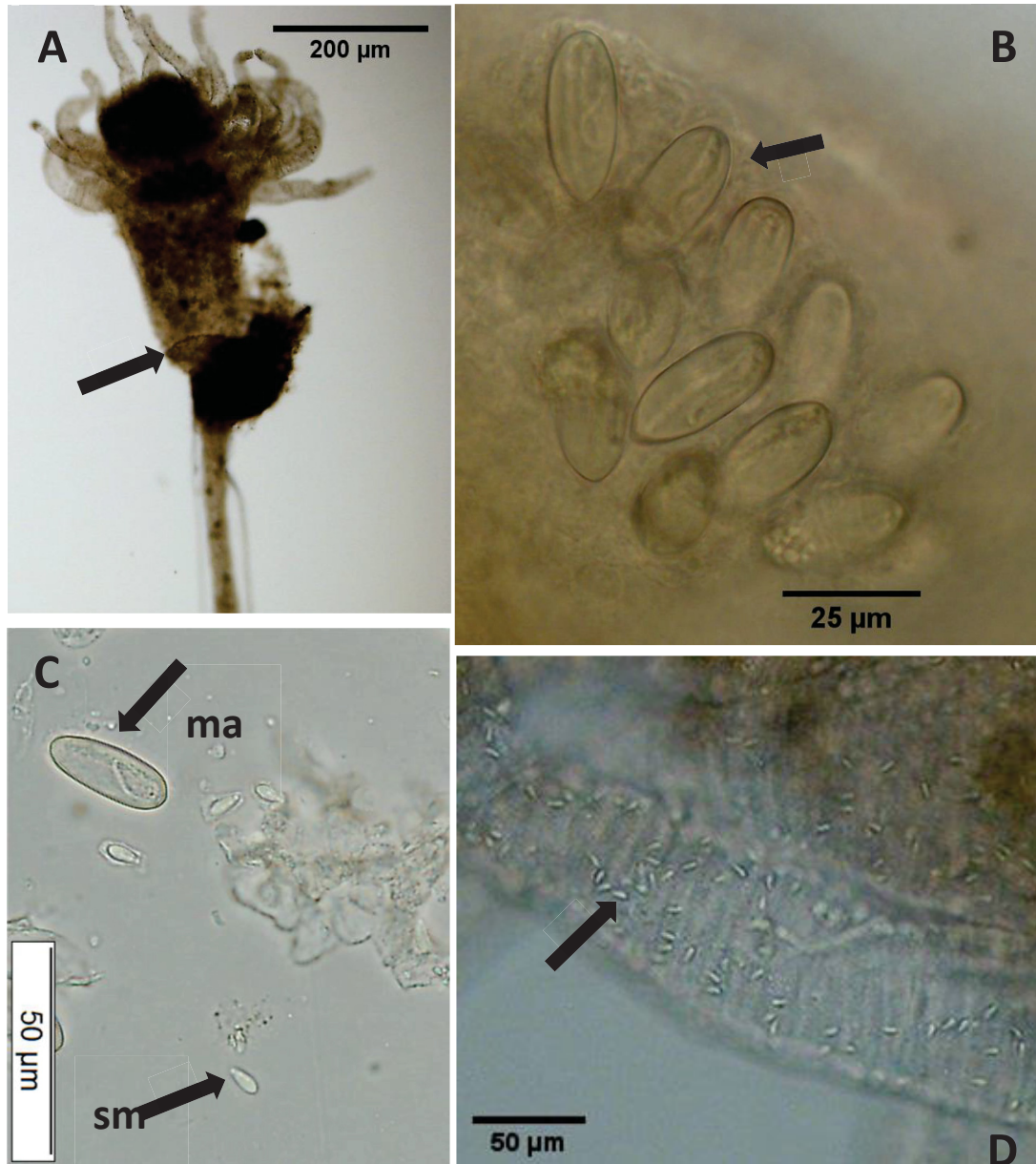


**Figure 12.** *Eudendrium klausi* A- hydranth with blue pigments (arrow) in tentacles, B- large microbasic euryteles abundant at the base of hydranth (arrow), C- undischarged large microbasic eurytele, D- nematocysts. **sm** = small microbasic eurytele, **dlm** = discharged large microbasic eurytele



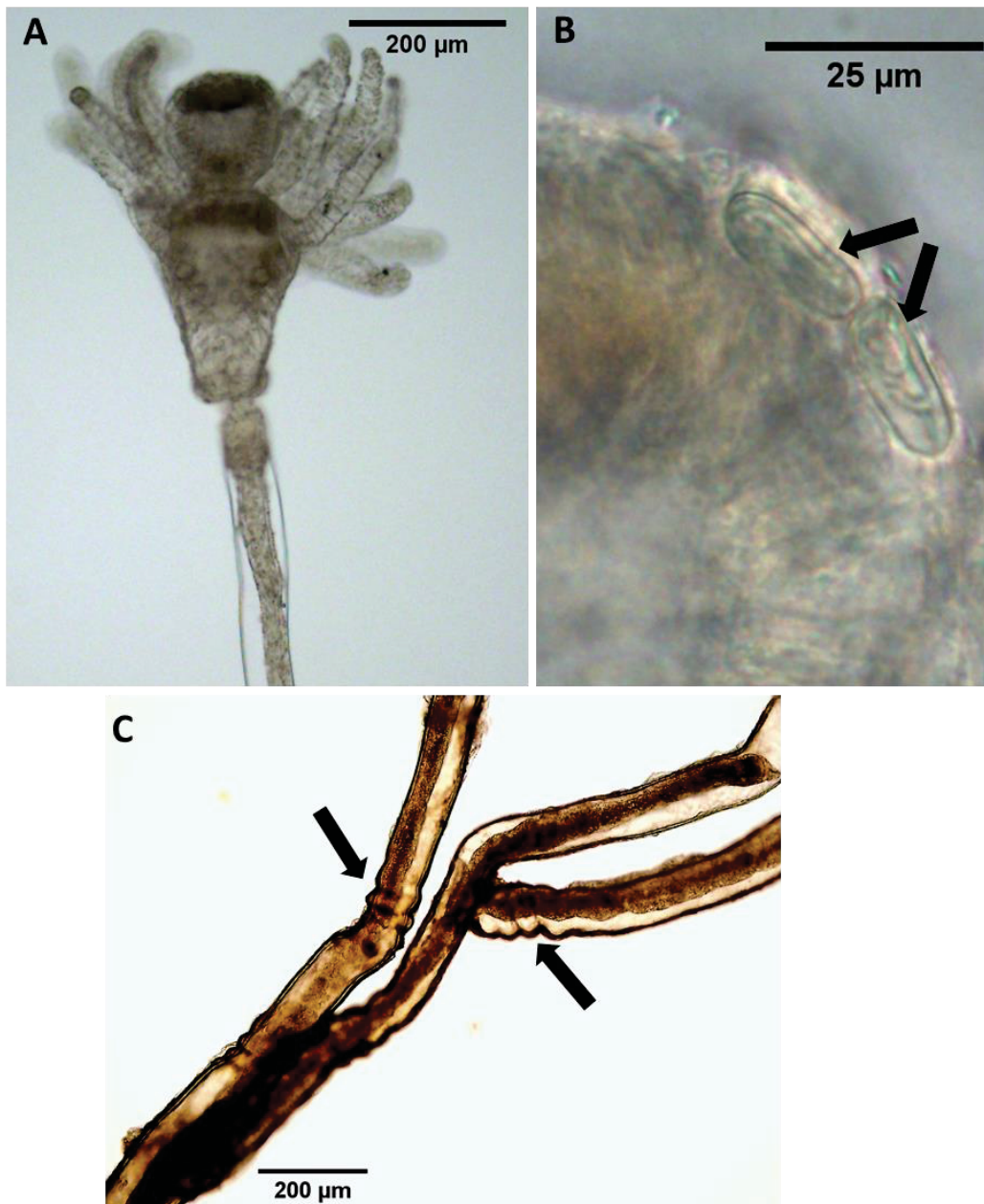


**Figure 13.** *Eudendrium moulouyensis*. A- Polyp, B- Zooxanthellae in tentacles (arrow), C- female gonophores (arrow), D- nematocysts, E- nematocysts in coenosarc of hydrocaulus. **sm** = small microbasic euryteles, **dlm** = discharged large microbasic euryteles. **sh** = shaft, **lm** = undischarged large microbasic eurytele, **zo** = zooxanthellae.

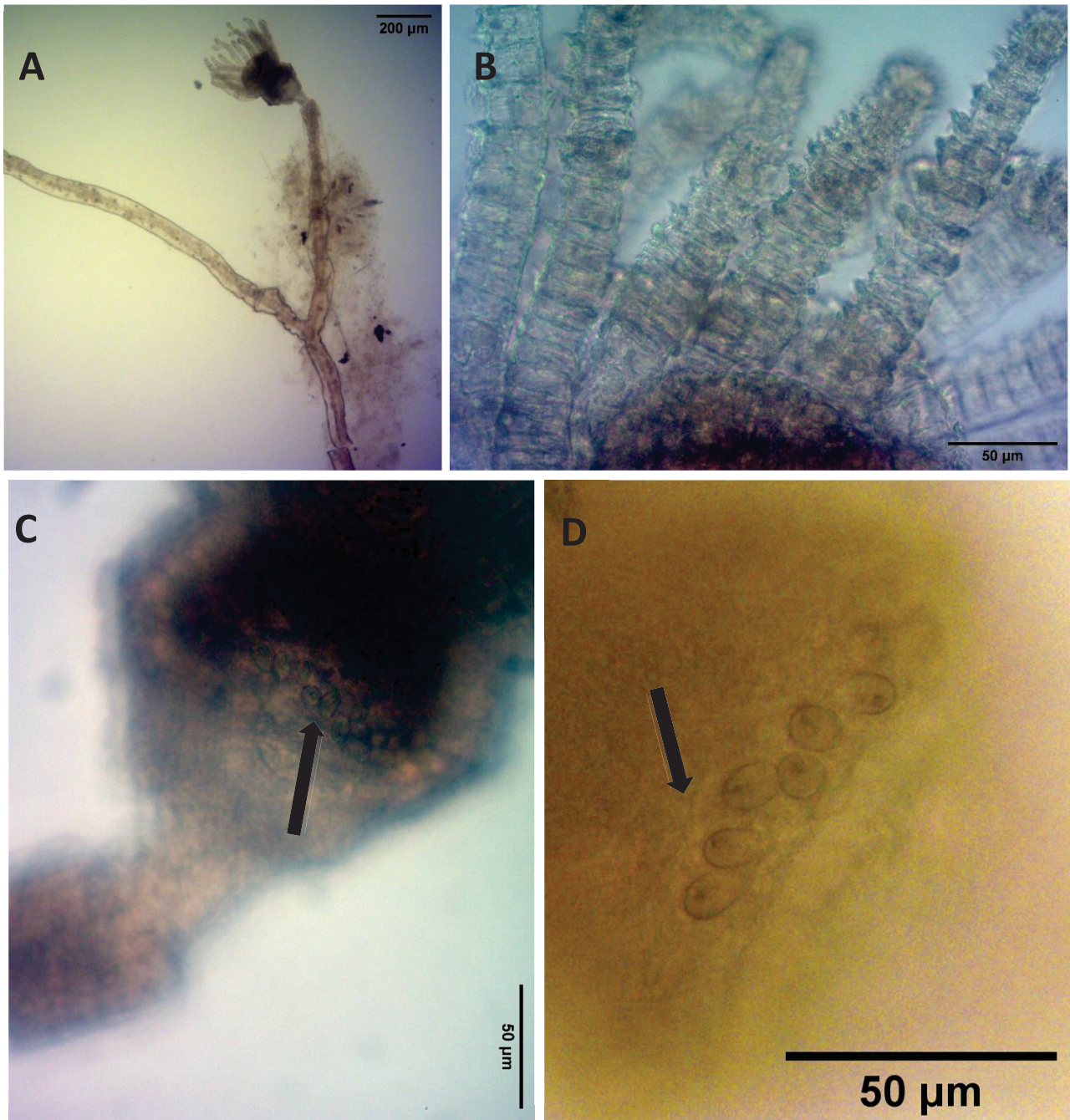


**Figure 14.** *Eudendrium bermudense*. A- hydranth with groove visible at the base (arrow), B- ring of macrobasic euryteles on hypostome, C- nematocysts, D- microbasic euryteles in tentacles (arrow). **ma** = macrobasic eurytele, **sm** = small microbasic eurytele



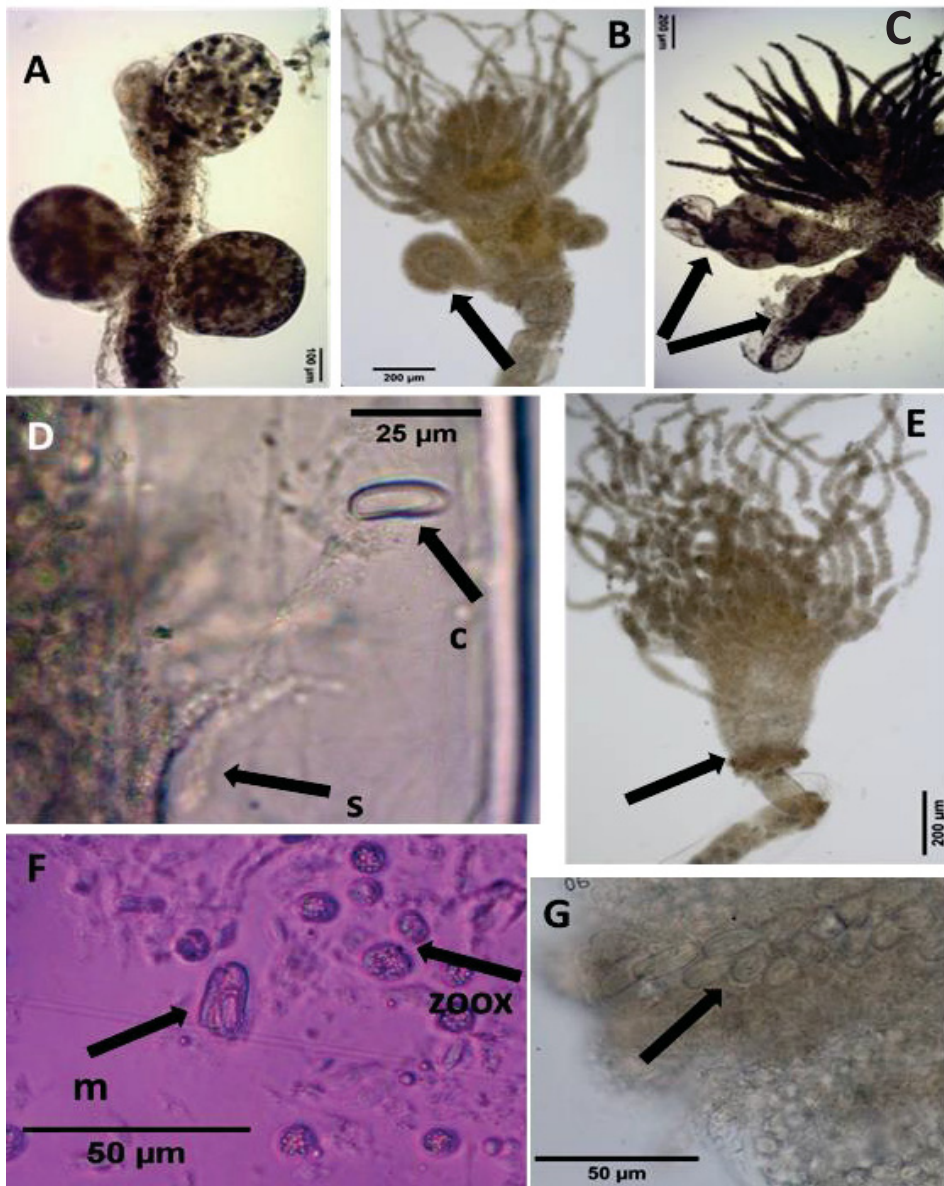


**Figure 15.** *Eudendrium album* A- hydranth, B- macrobasic euryteles on hypostome (arrows), C- detail of annulations on pedicels and hydrocaulus (arrows)

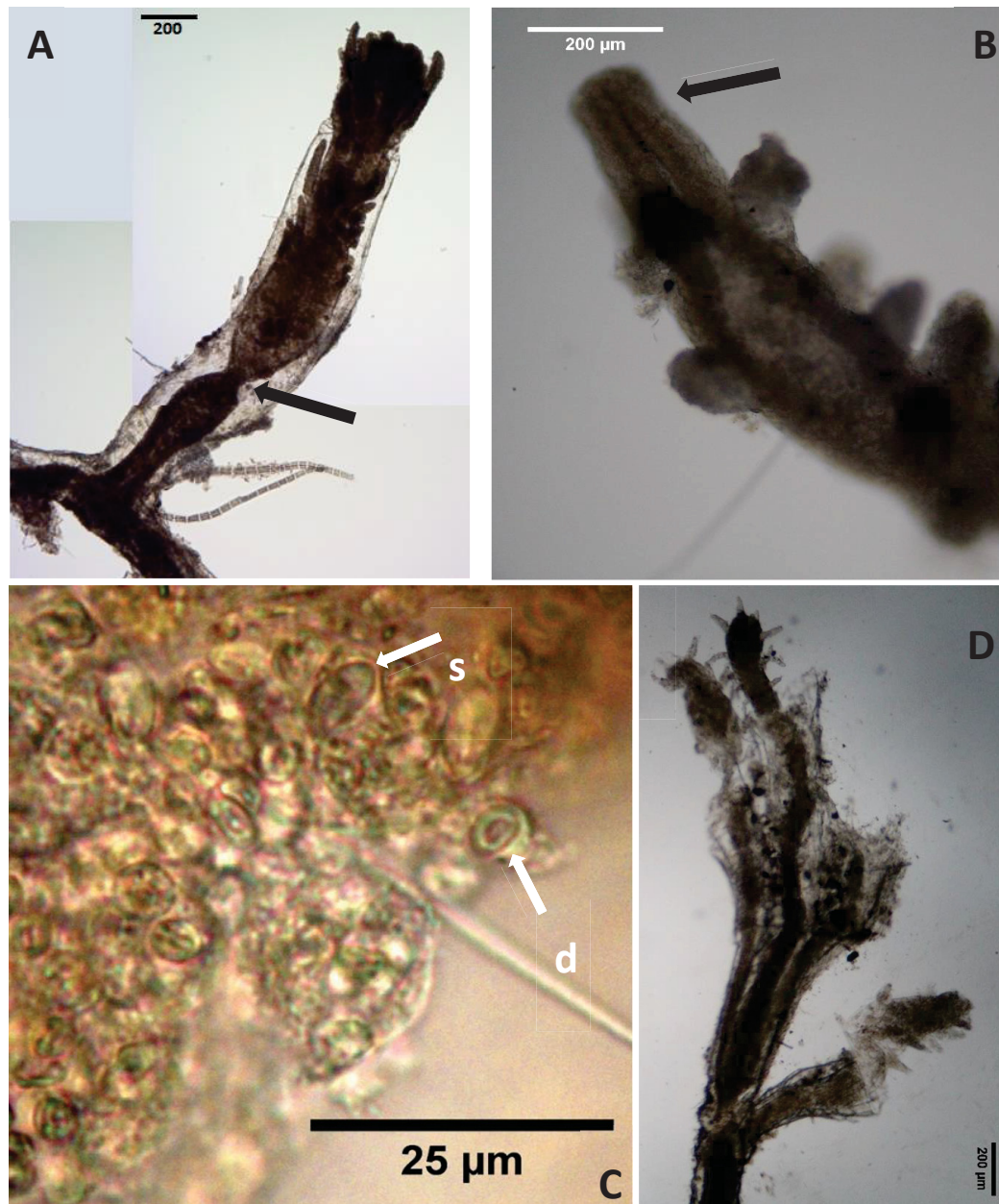


**Figure 16.** *Eudendrium* sp.1. A- Segment of hydrocaulus with one polyp, B detail of spiny aspect of tentacles, C and D- ring of nematocysts (large microbasic eurytheles) in the middle of hydranth body (arrow),



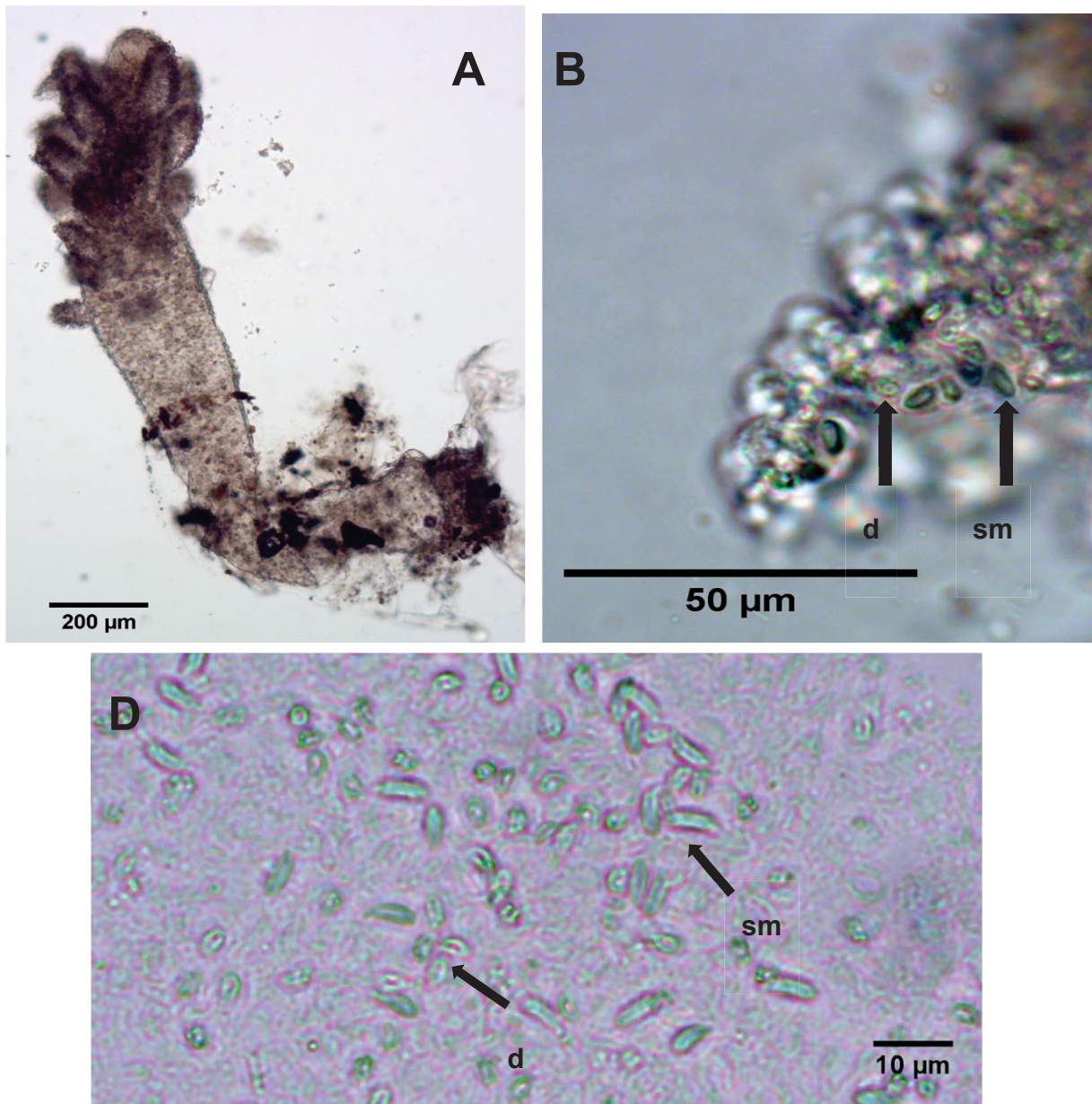


**Figure 17.** *Myrionema amboinense* A- Three mature female gonophores, B- hydranth with immature female gonophores (arrow), C- hydranth with male gonophores (arrows), D- discharged macrobasic eurytele in coenosarc, E- ring of macrobasic euryteles at the base of hydranth, E- undischarged macrobasic eurytele and zooxanthaellae. F- ring of macrobasic euryteles at hydranth base, detail (arrow). **c** = capsule, **sh** = shaft, **zoox** = zooxanthellae, **m** = macrobasic eurytele.

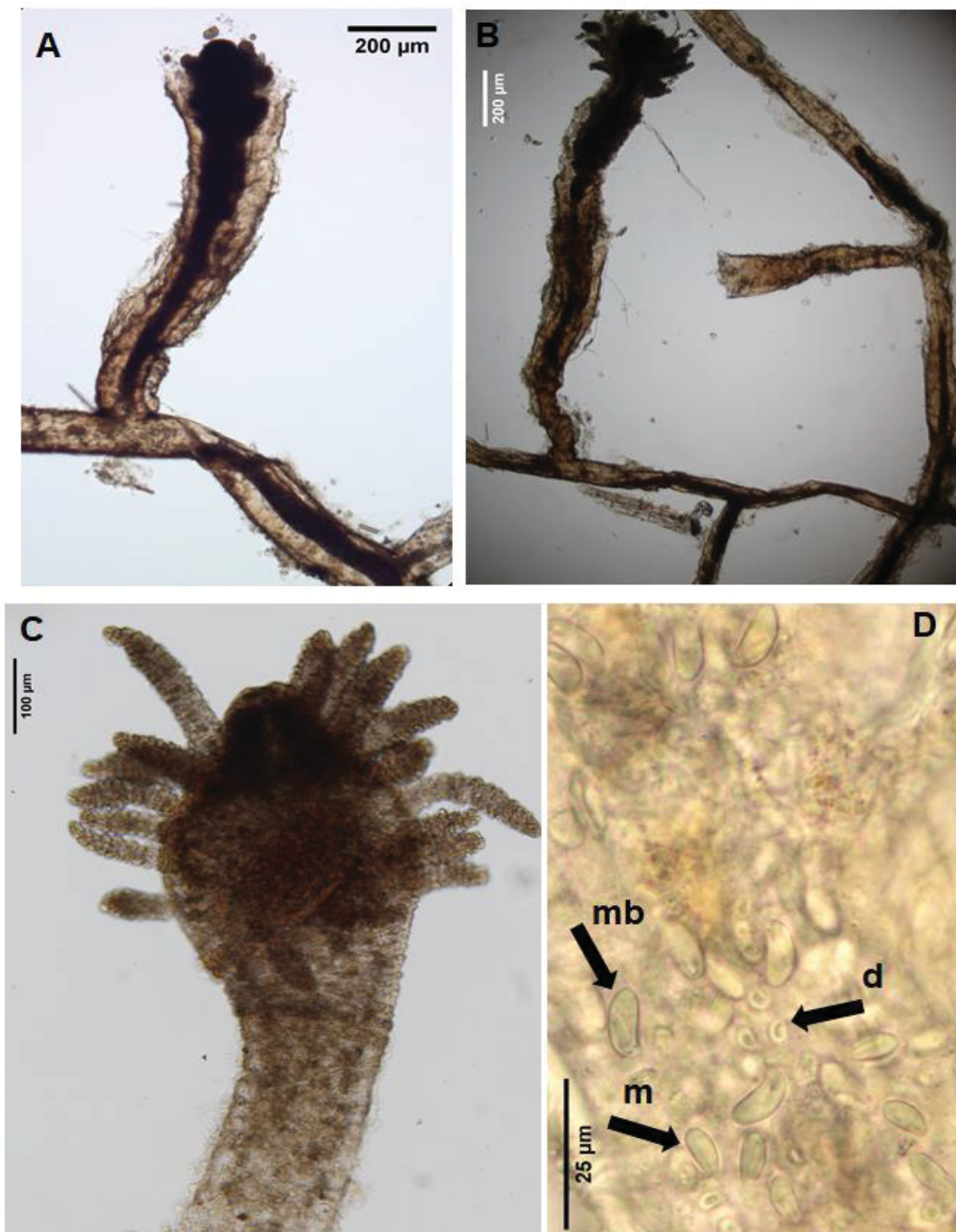


**Fig 18.** *Corydendrium parasiticum* A- stolonial polyp with detail of constriction in the base of hydranth (arrow), B- Detail of hypostome in relaxed polyp (arrow) C- nematocysts in tentacles, D- branched colony. **sm** = small microbasic eurytele **d** = desmoneme.

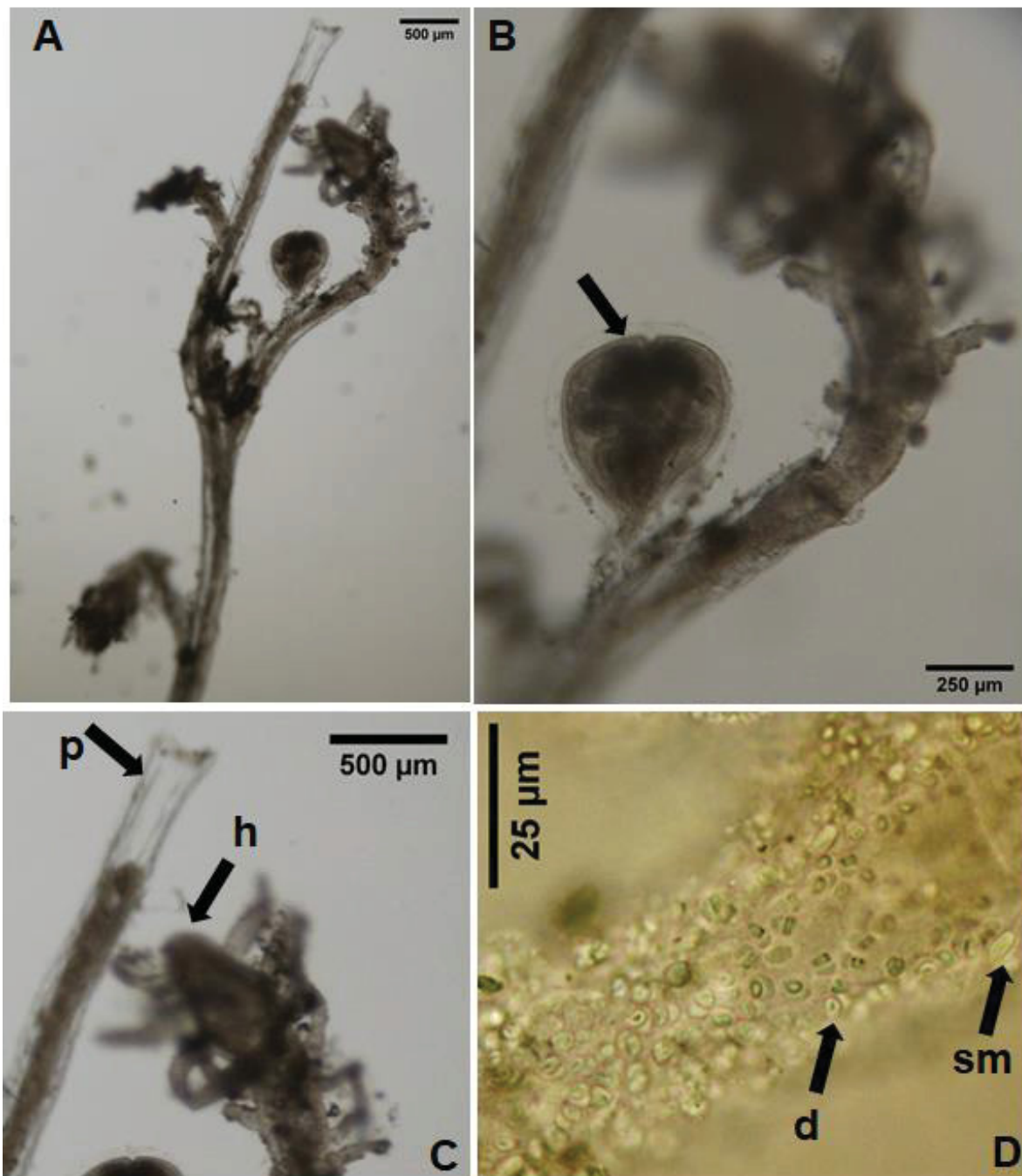




**Figure 19.** *Rhizogeton sterreri* A – stolonial hydrant, B – nematocyst in tentacles, C – nematocysts. **d** = desmonemes, **sm** = small microbasic euryteles.



**Figure 20.** *Rhizogeton conicum* A-B- colony fragment with hydranth and pedicels. C- relaxed hydranth, D- nematocysts. **m** = microbasic euryteles, **mb** = microbasic euryteles bean shape, **d** = desmonemes.



**Figure 21.** *Turritopsis nutricula* A- Colony with gonophore, B- detail of gonophore (arrow), C- hypostome and detail of the perisarc with two layers, D- nematocysts in tentacles.  
**p** = perisarc, **h** = hypostome, **d** = desmoneme, **sm** = small microbasic eurytele.

## **CAPÍTULO 2**

### **CONTAMINATION AND WATER QUALITY EFFECTS ON ATHECATE HYDROIDS (CNIDARIA, HYDROZOA, ANTHOATHECATA) DIVERSITY IN TROPICAL MARINE IMPACTED HABITATS.**

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## CONTAMINATION AND WATER QUALITY EFFECTS ON ATHECATE HYDROIDS (CNIDARIA, HYDROZOA, ANTHOATHECATA) DIVERSITY IN TROPICAL MARINE IMPACTED HABITATS.

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### Abstract

This study evaluated the species richness and frequency of occurrence of athecate hydroids and related them to the organic contamination gradient in two impact habitats of the marine platform of Cuba. Collections were made on Havana's coral reefs in March/2013 and on seagrass meadows in Villa Clara in May/2011, by SCUBA diving in seven sampling sites on each habitat, using the quadrat method. Eighteen species, twelve in coral reefs and seven in seagrass meadows were found. *Eudendrium carneum* (11.9 %) and *Coryne pusilla* (7.14 %) were the most frequent species on coral reefs, while *Myrionema amboinense* (26.1 %) and *Eudendrium moulouyensis* (17.8 %) were the most frequent in seagrass meadows. To test whether the gradient of contamination was a driver of variations in athecate hydroids diversity in the coral reef, these chemical organic contaminants were used: sterols (coprostanol, cholesterol, and stigmasterol), linear alkylbenzenes (LABs), polycyclic aromatic hydrocarbons (PAH), PAH from 4 to 6 rings, and aliphatic hydrocarbons (HA) including the unresolved complex mixture (UCM). In seagrass, environmental variables of water quality (depth, salinity, turbidity, temperature, pH, oxygen saturation (SO), dissolved oxygen (DO), chemical oxygen demand (COD)), and the impact of contamination as coast distance from shore to each sampling points (DistCoast) were tested. In Havana's coral reef, the best predictors for athecate hydroids richness were

coprostanol combined with Total Linear alkylbenzenes (cop+Total LABs), and with the unresolved complex mixture (cop+UCM). In seagrass meadows turbidity and distance from the coast marked the differences between sites. Dissolved oxygen was significant for the variation on hydroids assemblages. These results show that the distribution and diversity of the athecate hydroid assemblages were sensitive variables to the gradient of chemical markers of organic contamination by sewage, oil and detergents, in Havana reefs, and also sensible to physical factors of water quality conditions, as turbidity and dissolved oxygen, and to human impact expressed as the distance from the coast, in seagrass supporting once again the importance of this group as environmental indicators.

## 1. Introduction

Coral reefs and seagrass meadows are coastal ecosystems essential to the health of the biosphere (Costanza et al., 1997; Bjorn, 1997; Hemminga and Duarte, 2000), and for the sustainable development of coastal, mainly in the tropical region (Bjorn, 1997). They represent an important source of carbon storage and are high productive ecosystems in the world (Kennedy and Björk, 2009). Currently, pollution due to anthropogenic impacts in the marine environment is considered a world concern. Anthropogenic impacts are affecting these ecosystems in many places around the world, playing an important role in their decline (Bell 1992; Nyström et al., 2000; Green and Short, 2003; Ralph et al., 2007; Waycott et al., 2009; Fabricius, 2011, Walker et al., 2013, Unsworth et al., 2015; Jackson et al., 2014; Graham et al., 2015). One of the main causes of the degradation of coral reefs and seagrass meadows in the world is sedimentation (Nugues and Roberts, 2003). Land runoff and industrial or dredged effluents, cause an increase in sedimentation and turbidity, affecting the structure of benthic ecosystems (Roger, 1990; Ralph et al., 2007).

In Cuba more than 98 % of the edge of the marine shelf is constructed by coral reef formations (Alcolado, 2007; Lopez et al., 2012), and more than 50 % of the plains of the shelf are occupied by seagrass meadows (Lopez et al., 2012). Some of the most important sources of anthropogenic impacts that threaten these ecosystems in Cuba are the overfishing, organic pollution produced by sewage in coastal towns and from agricultural runoff. Coastal urbanization

causes pollution and deterioration of the quality of marine waters (Gobert et al., 2009). The increase of water turbidity and eutrophication, road constructions in the sea to communicate several keys to the main island, modify the hydrodynamics impacting the seabed in seagrass ecosystems and consequently the ecosystems biodiversity (Alcolado et al., 2007; Menéndez Carrera et al., 2015). On the other hand, overfishing is one of the main impacts on Cuban coral reefs (Gonzales-Diaz et al., 2018).

Hydroids (Cnidaria: Hydrozoa) are abundant components in the most common benthic ecosystems throughout the world like coral reefs, rocky shores, mangroves, and seagrass (Coma et al., 1999; Gili and Hughes, 1995; Di Camillo et al., 2017; Boero, 1981) where they are among the first organisms to colonize hard substrates and other organisms (Gili and Hughes, 1995). Hydroid's colonies with a modular and clonal formation give them a high phenotypic variability and an ability to unlimited growth (Marfenin, 1997) to form large assemblages (Di Camillo et al., 2017) and to occupy large areas. Their growth may vary in relation to environmental conditions (Gili and Hughes, 1995).

Between benthic hydroids there are two groups *Lepthothecata* (thecate) and “*Anthoathecata*” (athecate) morphologically mainly differentiated by the characteristic of the exoskeleton. From both, *Lepthothecata* are considered the most specious group (Maronna et al., 2016). Both groups are sensitive to several environmental factors (Mergner, 1987; Cabral, 2013). that significantly influence in assemblage species composition, diversity, and distribution. Between these factors we mention temperature, salinity, depth, substrate, light, and sedimentation (Calder, 1991; Gravier-Bonnet and Bourmaud; 2006; Cunha and Jacobucci, 2010; Ronowicz et al., 2013; Fernandez et al., 2014, 2015; Genzano et al., 2017).

Pollution is also one of the factors that influence the structure of hydroid communities (Boero, 1984), therefore, they are considered indicators of environmental quality in the marine environment (Mergner, 1977, 1987; Cabral, 2013; Megina et al., 2016; Castellanos-Iglesias et al., 2018; Yilmaz et al., 2020; Topcu et al., 2018; De Castro, 2020). One of the main stressor factors in coastal ecosystems is the pollution by sewage (Cabral and Martins 2018). Sterols are used as molecular markers to indicate this kind of pollution and continental material contributions (Derrien et al., 2017): cholesterol, classified as faecal sterol (Martins et al., 2018), is present in zooplankton and phytoplankton (Volkman, 2005) and stigmasterol determines the contributions

of marine organisms to the organic pull (Hudson et al. 2001). Related to sewage discharge there is the Linear alkylbenzenes (LABs) that are compounds used in the production of linear alkylbenzene sulfonate (LAS) surfactants for commercial detergents, they are frequently discharged via sewage and conserved in sediments for 10 to 20 years (Eganhous et al., 1983, Takada and Eganhouse, 1998; Martins et al., 2014). To evaluate organic contamination derived from fossil fuels, and related by-products were used the polycyclic aromatic hydrocarbons (PAHs) that are organic compounds derived from oil and from incomplete combustion of organic matter sources (Sette et al., 2013) and are ubiquitous in marine environments (Hylland 2006). and aliphatic hydrocarbons (AHs) (Dauner and Martins, 2015; Cardoso et al., 2016). Aliphatic hydrocarbons include the unresolved complex mixture (UCM) (Martins et al., 2018).

Studies of the athecate hydroid fauna of Cuba are scarce, with a slight increase in recent years and have been made in Coral reefs and seagrass meadows, and mainly directed at taxonomy or simple registers (Ortiz, 1995; Rosado, 2000 a, b; Ortiz, 2001; Lalana et al. 2001; Varela et al. 2005; Castellanos-Iglesias 2009; Varela and Cabrales 2010, 2012; Varela 2011), as opposed to the thecate hydroids, which have taxonomic studies with descriptions (Castellanos-Iglesias et al. 2011 and Castellanos-Iglesias, 2017). Studies regarding hydroids ecology for Cuban waters have received less attention (Castellanos-Iglesias et al., 2011, 2018; Castellanos-Iglesias, 2017). To start filling this gap, we planned this work with the objective to - Determine the structure, richness, and abundance (frequency of occurrence) of the athecate hydroids specifically: 1- Related to the variation in organic contamination gradient in Havana's coral reefs and 2-Related to the water quality conditions and contamination impact on seagrass ecosystem in Villa Clara.

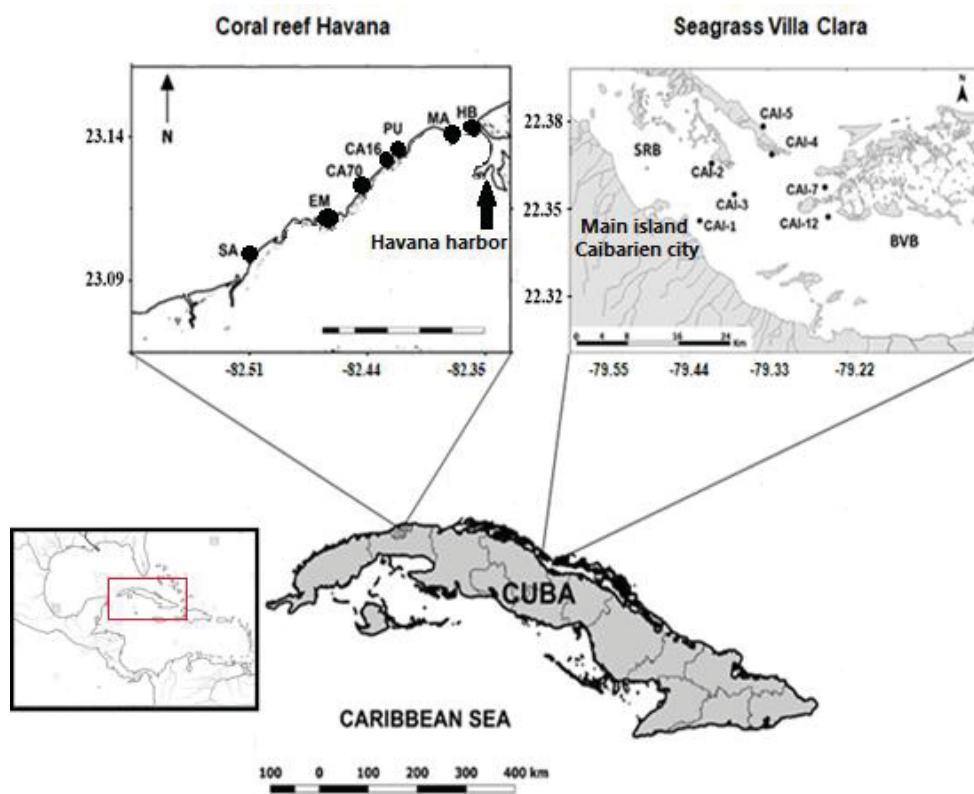
For these objectives we aimed to test the following hypothesis:

- 1- Because of the sensitivity of hydroids to contaminants, it is expected to find differences in diversity between sites along the Havana coral reef contamination gradient with a diminish of diversity in the most contaminated coral reef areas closer to the Bay of Havana.
- 2- In seagrass meadows, we hope to find differences in diversity of athecate hydroids between sites, expecting lower values at sites closer to the main island, where worst water quality conditions prevail indicating contamination.

## 2. Methods

### 2.1. Study areas

This study was conducted in two habitats located in the north coast of Cuba main island: the fringing coral reefs (CR) of the western coast of the Havana city, and the seagrass meadows of two shallow water bays, San “Juan de los Remedios” (SRB) and “Buena Vista” (BVB), in the north of the city of Caibarien, province of Villa Clara, central region of Cuba. The sampling sites have been chosen in relation with the contamination sources, the Havana harbor for coral reefs, and the proximity to the main island (Caibarien city) for the seagrasses (Fig. 1, Table 1). The impact of contamination in these ecosystems were evaluated in previous studies (Castellanos-Iglesias et al, 2018; Martins et al., 2018, Martinez-Daranas et al., data not published).



**Figure. 1.** Map of Cuba with the location of the studied areas and sampling sites (see Table 1 for abbreviations).

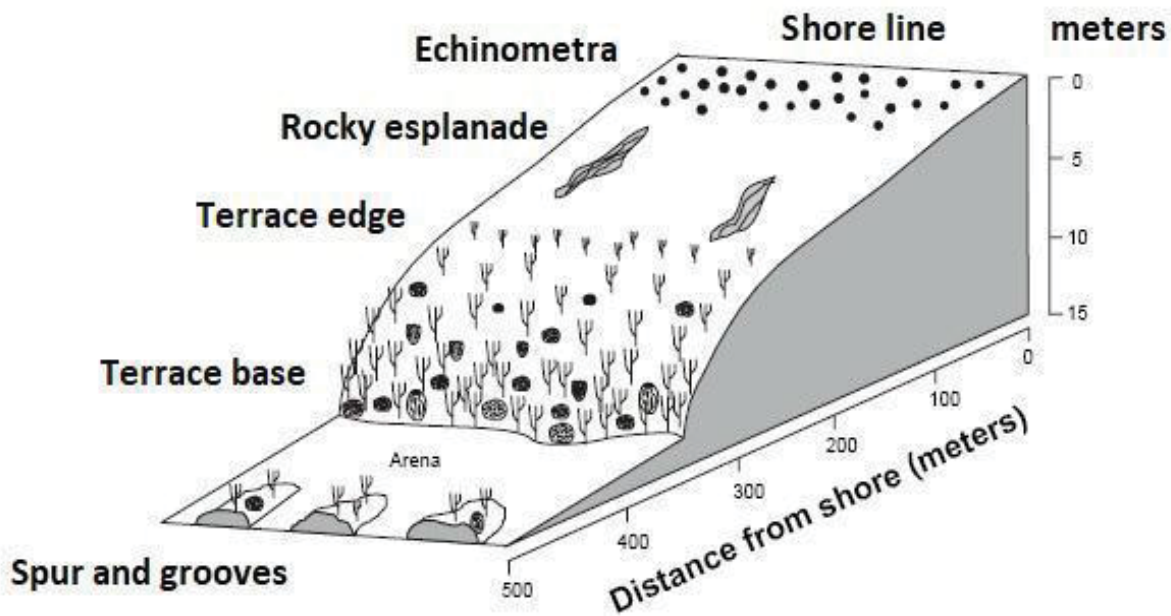
**Table 1. Localization of study areas and sampling sites, with detail of depth, and distance from main source and level of contamination.**

Study area	Sampling site	Lat (N)	Long (W)	Habitat	Water depth (m)	SC	Distance from SC (Km)
<b>Seagrass meadows ecosystem</b>							
VC	CAI-2	22 35 49	-79 23 98	SM	2	Mi	9.4
VC	CAI-3	22 33 31	-79 22 04	SM	2.5	Mi	8.9
VC	CAI-4	22 36 31	-79 19 30	SM	2.6	Mi	16.2
VC	CAI-5	22 37 97	-79 19 74	SM	2.8	Mi	17.7
VC	CAI-7	22 34 08	-79 15 04	SM	2.5	Mi	18.6
VC	CAI-1	22 28 56	-79 16 08	SM	3.1	Mi	2.5
VC	CAI-12	22 31 46	-79 14 46	SM	2.7	Mi	14.9
<b>Coral reef ecosystem</b>							<b>LC</b>
NH	HB	23 14 70	-82 35 86	CR	10	Hh	C
NH	MA	23 14 22	-82 37 00	CR	10	Hh	C
NH	ALM	23 13 69	-82 42 14	CR	10	Hh	MC
NH	CA16	23 12 85	-82 42 31	CR	10	Hh	MC
NH	CA70	23 12 56	-82 44 47	CR	10	Hh	NC
NH	EM	23 10 64	-82 46 75	CR	10	Hh	MC
NH	SA	23 09 33	-82 51 00	CR	10	Hh	NC

**Legend:** VC= Villa Clara, NH= North Havana; CAI= Caibarien Municipality; HB= Havana Bay; MA= Maceo; ALM= Almendares; CA16= Calle 16; CA70= Calle 70; EM= Emisario; SA= Santa Ana. CR= coral reef, and SM= Seagrass meadows; Mi= Main Island; Hh= Havana harvour; SC=Source of contamination, LC=Level of contamination, No contaminated (NC); Moderated contamination (MC); Contaminated (C).

### 2.1.1. Coral Reef - Havana

These coral reefs form a submarine terrace with a slope from approximately 8 m to 12–15 m depth, followed by a sandy plain. The terrace of about 300 m width extends parallel to the coastline (Aguilar et al., 2004) and can be divided into four distinct biotopes (Fig. 2).



**Figure 2.** Fringing coral reef in the study zone with its common bottom biotopes in the coast of Havana city (modified from Aguilar et al. 2004).

Seven sampling sites were distributed on the shallow terrace edge biotope, at 10 m depth, along 21 km of the Havana shore (from 23°08'49"N, 82°21'31"W to 23°04'25"N, 82°31'37"W). This coral reef is characterized by a high biodiversity of fish community, macrobenthic fauna and flora (Aguilar et al., 2004, Betancourt and González-Sansón, 2011; Acosta and De la Guardia Llansó, 2004; Rey-Villiers et al., 2020b, González-Díaz et al., 2018), despite having signals of chronic contamination (Castellanos-Iglesias et al., 2018 and Martins et al., 2018) which produce negative effects in these marine organisms.

In the coast of Havana, the coral reef is influenced by different sources of pollution, mainly from sewage and other pollutants such as detergents and oil. Historically, the major source of pollution into this area is the port of Havana (Martin et al., 2018, Aguilar et al. 2004, Duran et al.



2018), to which are added discharges from two rivers of polluted waters: Quibú River and Almendares River. Through analysis of the sterols in the sediment, it has been determined that there is a gradient of fecal contamination along the seven collection sites, where the closest site to the port of Havana (HB) is the most contaminated, and to the west direction the SA site is the least contaminated (Castellanos-Iglesias, et al., 2018)

### *2.1.2. Seagrass Meadows-Villa Clara*

The seagrass meadows are in two shallow bays, Buena Vista and San Juan de los Remedios, in the north of Caibarien city, province of Villa Clara. In these bays the exchange of water with the ocean is limited, and their land runoff is low because the few major rivers have been dammed (Claro et al. 2001). The seagrasses grow in a shallow marine platform composed mainly of silt clay sediments of 2 to 5 m deep (Martinez-Daranas et al., 2007). This is an especially important economic lobster and commercial fishes fishing zone. This area of 3.414 km<sup>2</sup> is part of the Savanna-Camagüey Ecosystem (SCE) which extends along 465 km of the north coast of Cuba and is composed of 2.515 keys. For more details of the study area see (Castellanos-Iglesias, 2017).

## *2.2. Field sampling*

### *2.2.1. Hydroid's collection*

Collections in the Havana coral reef were made in March/2013, by SCUBA diving, using the quadrants, at 10 m deep. In each of the seven sampling sites (Fig.1, Table 1), two 10 m transects were positioned parallel to the coastline. Three squares of 25 x 25 cm were randomly placed along each transect, summing 6 quadrants per site and a total of 42 in this habitat. All fauna and flora within each quadrant were collected with a spatula, put into a bag with local sea water, anesthetized with menthol and after about 2 hours were fixed in 4 % formalin.

The same technique of collections was used in the seven sampling sites (Fig.1; table 1) of the seagrass meadows, in May/2011, between 2 and 3.1 m deep. Only the sample design was a little different: two crossed transect lines 15 m long were positioned in each site. Six quadrants 25 x 25 cm were positioned along each transect, 5 m from each other, summing 12 sampling units per site and a total of 84 in this habitat. All the macrophytobenthos were collected in each



square to analyze the presence of hydroids. The samples were treated in the same way as for reefs.

### 2.2.2. Organic contaminants on coral reef's sediment and water quality on seagrass meadows

The analytical method for organic markers determination in marine sediments was based in Wisnieski et al. (2016). One surface sediment sample was collected per site using a stainless-steel pickup, then placed in aluminum foil, oven dried ( $<40^{\circ}\text{C}$ , 48 h), homogenized in a mortar and stored in glass bottles for analysis (Castellanos-Iglesias, 2017). Different chemicals organic contaminants were analyzed (Martins et al., 2018), as sterols (coprostanol, cholesterol, and stigmasterol), linear alkylbenzenes (LABs), polycyclic aromatic hydrocarbons (PAH), PAH from 4 to 6 rings, and aliphatic hydrocarbons (HA) including the unresolved complex mixture (UCM)., to test whether the gradient of contamination was a driver of variations in athecate hydroids diversity in the coral reef (Table S1).

In each sampling site of the seagrass meadows, different environmental variables have been measured as contamination indicators (Castellanos-Iglesias et al., data not published): coast distance (DistCoast) (km), depth (m) measured with diving consoles, turbidity (FTU) with a turbidimeter HANNA-HI 93703-11 of a precision of  $\pm 0.01$  FTU, salinity (ups), pH, oxygen saturation (%) (SO) and dissolved oxygen (DO) ( $\text{mg.L}^{-1}$ ) recorded with a probe HANNA HI-9828, to determine the chemical oxygen demand (COD) ( $\text{mg.L}^{-1}$ ), according to the analytical procedures of FAO (1975). Samples of the surface seawater were stored at  $4^{\circ}\text{C}$  and frozen at  $-20^{\circ}$ . Surface and bottom water temperature ( $^{\circ}\text{C}$ ) were recorded *in situ* (Table S2).

### 2.3. Laboratory analysis

Hydroids were separated from the substrates under a stereomicroscope Leika. The samples were analyzed and identified under a biological microscope OLYMPUS-BX50 and AxioLab A1 ZEISS, both equipped with cameras Olympus DP 72 and AxioCam ERc 5s, respectively, linked to the imaging software ImageJ (Schneider et al. 2012) The species identification follows mainly the taxonomic studies of Cuba, Caribbean, and tropical region of the Western Atlantic (Calder, 1988, 2010, 2013; Galea 2008, 2013; Puce et al., 2005 a and b) and of other regions and seas (Kelmo and de la Santa-Isabel, 1998; Marques, 1993, 2000;

Schuchert, 2001, 2004, 2005, 2006, 2007, 2008 a and b, 2009, 2012; Bouillon et al., 2004, De Vito et al, 2008).

## 2.4. Data analyses

### 2.4.1. Hydroid assemblage's structure

With a matrix of presence and absence of hydroids in each habitat, each site and replica (quadrants), the species richness  $S$  per site and habitat and the percentage of frequency of occurrence of each species also per site (FOS %) and habitat (FOH %) were calculated. FOS % was calculated as the number of replicates in which each species appeared, divided by the total number of replicates of each site  $\times 100$ . FOH % was the number of replicates in which each species appeared, divided by the total number of samples of each habitat  $\times 100$ . The frequency of all athecate per site and habitat were also calculated.

### 2.4.2. Modeling environmental covariates vs spatial variations of hydroids diversity

A model selection approach using generalized linear models (GLMs) were used for the two habitats to determine the relative importance (RI) of organic contaminants on sediments and water quality as covariates predictors of the species richness of athecate hydroids as response variables. A selection of a family distribution was done by visual inspection of data distribution, using all models containing all covariates. The family Poisson was the candidate selected to fit the models (Burnham and Anderson, 2002). The multicollinearity between covariates was tested by the variance inflation factor (VIF), less than 10, with package *car* (Fox and Weisberg, 2011). Applying a multi-model inference for model selection that better explained the response variable (species richness), all possible combinations to determine the importance of environmental factors were calculated. For this was used the functions `model.sel` and `model.avg` (package MuMIn 1.12.1 (Barton, 2015)). Models were ranked by AICc (Akaike's Information Criterion) and delta AIC values (Zuur et al., 2009). Akaike weights (AICw) were used and interpreted as the relative probability in which a particular model would have the best fit for another set of data, drawn from the same underlying processes.

To determine the spatial variability of species composition was applied multivariate analysis for each habitat from a resemblance matrix (Presence/Absence) expressed on dissimilarity measure of Jaccard index among sites.

To calculate the beta diversity between sites of each studied area (defining beta diversity as the degree of change in species identities between sample units in each spatial scale (Whittaker 1960, 1972), a PCO (principal coordinate analysis) (Anderson and Willis, 2003) was applied to the spatial distribution data, based on the Jaccard resemblance matrix, separately for both areas.

We did a PERMANCOVA (PRIMERv6, (Clarke and Gorley, 2006) to determine the relation of the environmental independent variables with the biological community, using a one-way scale design with sites as a fixed factor and 9999 permutations. The permutation method was the permutation of residuals under a reduced model (Anderson, 2001; McArdle and Anderson, 2001), and with the selection of Type I (sequential) of the sum of squares (SS). Before the analysis, all covariates were normalized to avoid the differences of measurements (different units and scales e.g.,  $\mu\text{m}$ , ups, %,  $\text{mg.L}^{-1}$ ,  $^{\circ}\text{C}$ , m). Pearson's correlation coefficient was used to assess correlations between all pairs of covariates using a threshold of 0.95 (Clarke and Ainsworth, 1993).

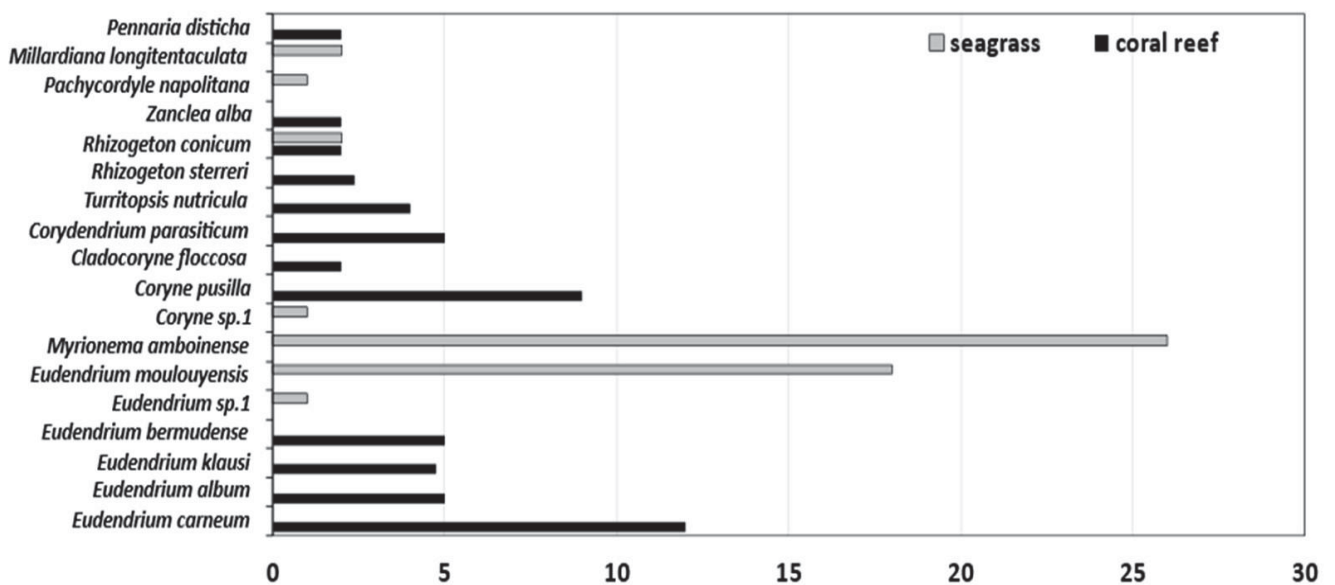
A canonical analysis of principal coordinates CAP (Anderson and Willis, 2003) was applied for two habitats to detect the effect of environmental variables on the Beta diversity multivariate dispersion of athecate hydroid assemblages between sites. This method shows a constrained ordination for any distance or dissimilarity measure. All these analyses were performed with the PRIMER-E software (Clarke and Gorley, 2006). The presence-absence matrix and the environmental variables matrix were multiple correlated with the canonical axes, to identify the individual contribution of species and covariates to the multivariate patterns of the dispersion of data (Anderson and Willis, 2003). The association between the two first axes of the CAP and the vectors were measured with multiple correlation coefficient.

### 3. Results

#### 3.1. Hydroid assemblage's structure in Havana coral reef and Villa Clara seagrass meadows

In both habitats, coral reef and seagrass meadow, eighteen morphotypes were found in 126 sampling units (quadrants), 42 in coral reefs, and 84 in seagrass meadows. Sixteen were identified to species level and two until genera level (*Coryne* sp.1 and *Eudendrium* sp.1). In coral

reefs twelve species were recorded and seven in the seagrass meadows. In the coral reef, *Eudendrium carneum* and *Coryne pusilla* had the most frequency of occurrence, 11.9 %, and 7.14 % respectively. In seagrass meadows *Myrionema amboinense* had the highest frequency of occurrence (26.1 %), followed by *Eudendrium moulouyensis* (17.8 %) (Fig. 3, Table 3 and 6).



**Figure 3.** Frequency of occurrence (%) of athecate hydroids, species composition in coral reef (42 quadrants) in Havana and seagrass meadows (84 quadrants) in Villa Clara, Cuba.

Table 3. Species richness (S) by site, absolute frequency (FA) and percentage of frequency of occurrence (%FO) of each species by site and by habitat in Coral reefs of Havana. CR= Coral reef; HB= Havana Bay; MA= Maceo; ALM= Almendares; CA16= Calle 16; CA70= Calle 70; EM= Emisario; SA= Santa Ana.

Taxons	Sites													
	HB		MA		ALM		CA16		CA70		EM		SA	
	FA	%FO	FA	%FO	FA	%FO	FA	%FO	FA	%FO	FA	%FO	FA	%FO
<b>Suborder Capitata</b> Kühn, 1913														
Family Cladocorynidae Allman, 1872														
<i>Cladocoryne floccosa</i> Rotch, 1871	0	0	0	0	1	16,67	0	0	0	0	0	0	0	1
Family Corynidae Johnston, 1836														
<i>Coryne pusilla</i> Alder, 1856	1	16,67	0	0	0	0	1	16,67	1	16,67	1	16,67	1	16,67
Family Zancleidae														
<i>Zanclea alba</i> (Meyen, 1834)	0	0	0	0	0	0	1	16,67	0	0	0	0	0	1
Family Pennariidae McCrady, 1859														
<i>Pennaria disticha</i> Goldfuss, 1820	1	16,67	0	0	0	0	0	0	0	0	0	0	0	1
<b>Suborder Filifera</b> Kühn, 1913														
Family Eudendriidae L. Agassiz, 1862														
<i>Eudendrium carneum</i> Clarke, 1882	0	0	1	16,67	0	0	3	50	1	16,67	0	0	0	5
<i>Eudendrium bermudense</i> Calder, 1988	2	33,33	0	0	0	0	0	0	0	0	0	0	0	2
<i>Eudendrium album</i> Nutting, 1898	1	16,67	0	0	0	0	0	0	1	16,67	0	0	0	2
<i>Eudendrium klausii</i> Puce, Cerrano, Marques and Bavestrello, 2005	0	0	0	0	0	0	1	16,67	1	16,67	0	0	0	2
Family Oceaniidae Eschscholtz, 1829														
<i>Corydendrium parasiticum</i> (Linnaeus, 1767)	0	0	0	0	2	33,33	0	0	0	0	0	0	0	2
<i>Rhizogeton sterreri</i> (Calder, 1988)	1	16,67	0	0	0	0	0	0	0	0	0	0	0	1
<i>Rhizogeton conicum</i> Schuchert, 1996	1	16,67	0	0	0	0	0	0	0	0	0	0	0	1
<i>Turritopsis nutricula</i> McCrady, 1857	0	0	0	0	0	0	0	0	0	0	1	16,67	0	1
<b>FA Athecate hydroids</b>	<b>7</b>		<b>1</b>		<b>3</b>		<b>6</b>		<b>4</b>		<b>2</b>		<b>1</b>	
<b>S Athecate hydroids</b>	<b>6</b>		<b>1</b>		<b>2</b>		<b>4</b>		<b>4</b>		<b>2</b>		<b>1</b>	

### 3.2. Hydroid species richness and frequency distribution in Havana coral reefs

The twelve species found in coral reefs are classified in six families, Oceaniidae, Corynidae, Zancleidae, Pennariidae, Cladocorynidae, and Eudendriidae, and six genera. Eudendriidae and Oceaniidae were the best represented families, the first with one genus and four species and the second with three genera and four species (Table 3).

Species richness analysis showed that HB was the site with more species richness (6) followed by CA70 and CA16 each one with four species (Table 3, Fig. 3). In HB, the most frequent species was *Eudendrium bermudense* (33,3 %). In MA and ALM, only one species was found, *E. carneum* (16.67 %) and *C. prasilicum* (33.3 %) respectively. In CA,16 four species appeared being *E. carneum* the most frequent (50 %). Four species were also found in CA70, all of them with the minimum value of percentage of occurrence. In EM, two species were found both with the minimum percentage of occurrence (16,67 %). In SA only one species was found (16.67 %).

### 3.3. Relationship between hydroid richness and organic contamination markers

On relating species richness of athecate and the organic contaminants (see Table S1), 271 generalized linear models (GLMs) were created of which six models had the best fits for richness (S) and were also selected by delta AIC value (Table 4). As a result of the global analysis, the most explanatory model was M1 with the variable PAHs from 4 to 6 rings. The second model was M2 with the combination of cop and Total LABs, being the latter one significant for p-value = 0.027. As a result of the average model, M3 was the most explanatory by the variables chol\_e and stig, followed by the M4 and M5 models including cop significantly combined with the contaminants unresolved complex mixture (UCM) (p-value = 0.012) and Total AHs (p-value = 0.020). The Total LABs contaminant was significant (p-value = 0.048) for the M6 model with chol-e, stig, and Total AHs.

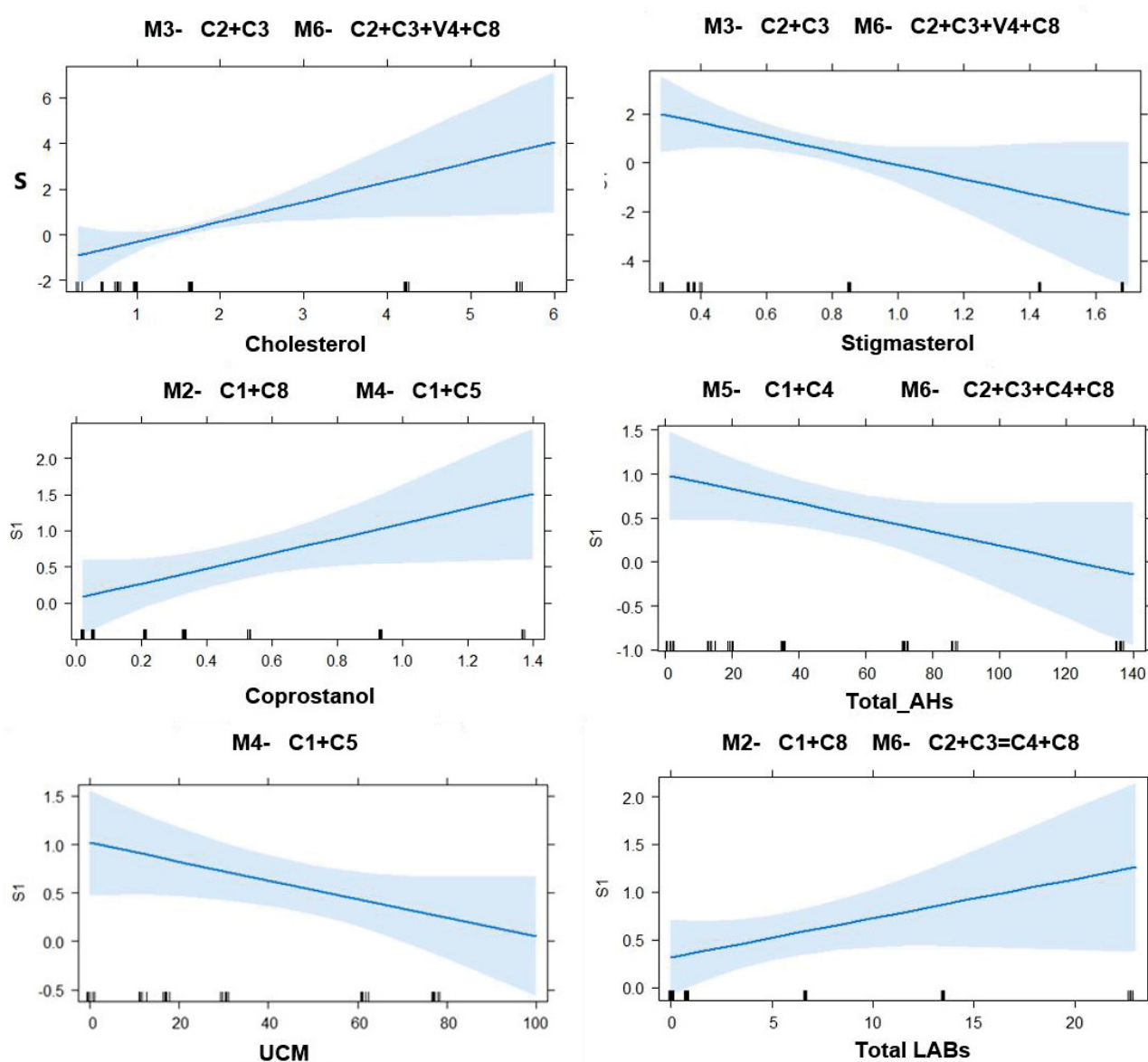
Table 4. Athecate hydroids in the coral reefs. Best-fit models (organized from the most to the least explanatory) obtained with global and average model analyses from GLM and application of Poisson distribution, ordered by delta AIC value and relative importance (RI) of each selected predictor variable for species richness.

Response Variables	Best fit models	Distribution family	df	AICc	Delta	AICw	Predictors	p-value	RI
Species richness global model	M1 (S_At ~ PAHs_4_6)	Poisson	4	102.8	<b>0.00</b>	0.030	PAHs_4_6	0.334	0.39
	M2 (S_At ~ cop + Total LABs)		4	103.5	0.68	0.02	cop	0.091	0.39
							<b>Total LABs</b>	<b>0.027*</b>	0.32
Species richness conditional model	M3 (S_At ~ chol_e + stig)	Poisson	4	102.83	<b>0.00</b>	0.03	chol_e	0.807	<b>0.51</b>
							stig	0.844	0.45
	M4 (S_At ~ cop + UCM)		4	103.51	0.68	0.02	cop	0.147	0.39
							<b>UCM</b>	<b>0.012*</b>	0.43
	M5 (S_At ~ cop + Total AHs)		4	103.69	0.86	0.02	<b>Total AHs</b>	<b>0.020*</b>	0.42
	M6 (S_At ~ chol_e + stig + Total AHs + Total LABs)		6	104.26	1.43	0.01	<b>Total LABs</b>	<b>0.048*</b>	0.32

Models had the highest (AIC) Akaike's Information Criterion and lowest delta and Akaike weight (AIC w). S= species richness of hydroids, M= Modelo, cop= coprostanol, chol\_e= cholesterol, stig= stigmaterol, UCM=Unresolve complex mixture, LABs = Linear alkylbenzenes, AHs= Aliphatic hydrocarbons, PAHs= Polycyclic aromatic hydrocarbons, RI= relative-importance of each selected of each predictor, which on a scale from 0 to 1, parameters are highlighted in bold. \* means,  $p < 0.001$  \*\*\* 0.01 \*\*\*\* 0.



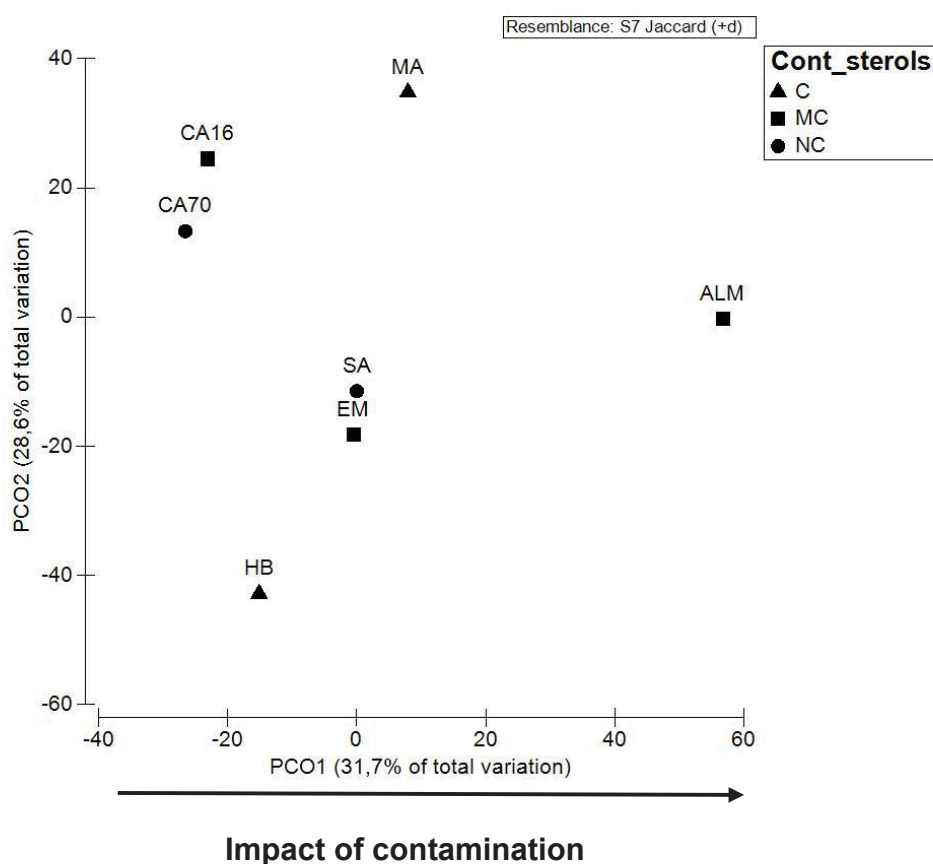
Between all contaminant predictors variables, the cholesterol showed the highest RI (RI = 0.51). Athecate hydroids richness increased with the increment of the cholesterol, coprostanol, and Total LABs concentrations and decreased with stigmasterol, Total AHs, and UCM concentrations. (Fig. 4).



**Figure 4.** Effect of organic contaminants on species richness (S) of athecate hydroids. M= model, Sterols: (C1= coprostanol, C2= cholesterol, C3= stigmasterol), C4= Total\_Ahs, C5= UCM, and C8= Total\_LABs.



A PCO analysis helped to explore the multivariate distribution data of the athecate hydroids richness related with the contamination impact determined by faecal sterols in the sediments. The first two principal components account for about 60 % of the total variation of the species composition, 31.7 % explained by the PCO1 and 28.6 % by PCO2 axes (Fig. 5), indicating a slightly variation in the dispersion between sites for species richness. PCO1 showed a slightly separation between sites less impacted (CA70-NC and CA16-MC) of those more impacted (MA-C and ALM-MC) by the contaminants.



**Figure 5.** PCO ordination of athecate hydroids in coral reefs of Havana, based on presence-absence data and Jaccard resembles matrix showing relationship between structure assemblage and contamination level between sites: C=contaminated sites (HB and MA), MC=moderate contamination (ALM, CA16 and EM), and NC=not contaminated (CA70 and SA). Site abbreviation in table 1

**Table 5. Multivariate analysis of variance PERMANCOVA to compare sites with the covariates (organic contaminants). \*=indicate statistical significance at the 95 % level ( $p < 0.05$ ). Component of variation (ECV) MC-Monte Carlo was applied because of the unique permutation less than 100.**

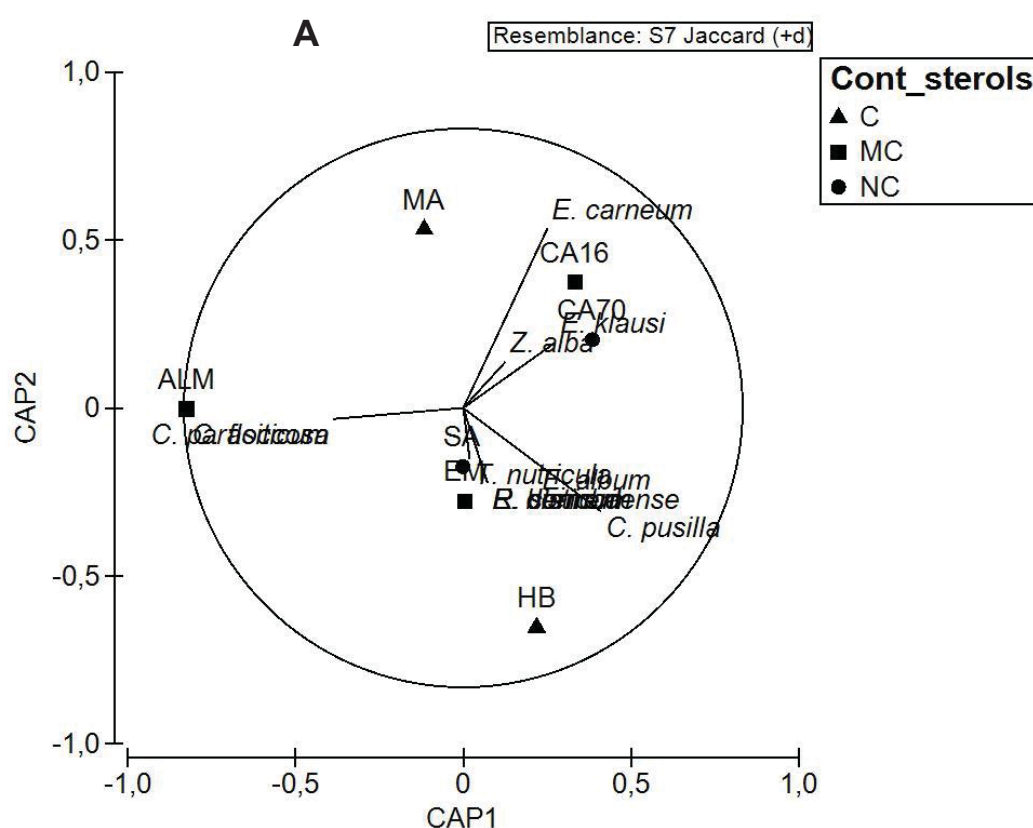
**Table 5. PERMANCOVA results:**

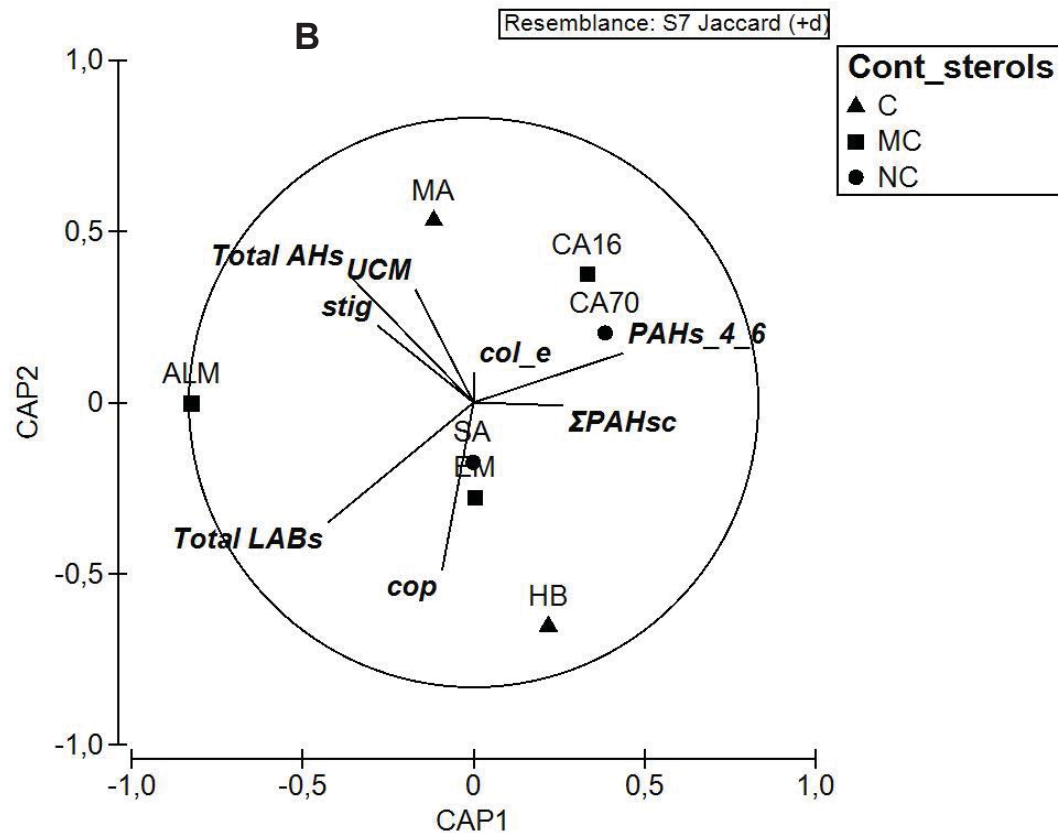
Covariates	df	SS	MS	Pseudo-F	P(perm)	perms	ECV (%)
Coprostanol	1	1464,3	1464,3	1,3308	0,2273	9940	0.015
Cholesterol	1	1637,9	1637,9	1,4885	0,1617	9948	0.022
Stigmasterol	1	1922,1	1922,1	1,7468	0,1017	9944	1.9
Total AHs	1	1678,7	1678,7	1,5256	0,1602	9935	0.07
UCM	1	1723	1723	1,5659	0,1444	9932	109.7
ΣPAHsc	1	698,49	698,49	0,63479	0,7067	9931	11.75
PAHs 4-6	0	0		No test			No test
<b>Total LABs</b>	0	0		No test			No test

**Legend: CM=Unresolve complex mixture, AHs= Aliphatic hydrocarbons, ΣPAHs= Polycyclic aromatic hydrocarbons summatory, PAHs= Polycyclic aromatic hydrocarbons, LABs = Linear alkylbenzenes.**

CAP analysis showed 50 % of the total multivariate constraint spatial ordering (50 %) for the athecate hydroids assemblages in coral reefs. CAP1 explained 40 % of the highest distribution of data and CAP2 explained only 10 % (Fig. 6A). Based on the group of sites related to the level of contamination by sterols (C, MC, NC), ALM (MC) had a marked difference from the other sites with a negative correlation in CAP1 axis. *Coryne pusilla* had the highest correlated value with the canonical CAP1 axis (0.49). It appeared in all sites except MA and ALM and showed a marked position in relation to HB (C). *Zanclaea alba* and *Eudendrium klausii* were positively correlated with the two axes as well as the sites CA16 (MC) and CA70 (NC). *E. carneum* had a positive correlation with MA (C), the most contaminated site, occupied only by this species. *E. carneum* contributed with the similarity position between sites CA16 (MC) and CA70 (NC), *E. carneum*, *E. klausii*, and *Z. alba* had a positive correlation with both axes and the highest value was for *E. carneum* with CAP2 (0.64). *Cladocoryne floccosa* and *Corydendrium parasiticum* had a high negative correlation with CAP 1 axis (-0.45), both exclusive of ALM site. The dispersion of the species *Rhizogeton sterreri*, *Rhizogeton conicum*, *Eudendrium bermudense*, *Pennaria dicticha*, *Turritopsis nutricula* and *Eudendrium album* were almost equally influenced by the canonical correlation of both axes. All of them had a negative correlation with CAP2 and better positively correlated with CAP1. All these species characterized HB (C) site, except for *T. nutricula* which is exclusive of EM (C).

Cop and Total LABs had a negative correlation with CAP1 and CAP2 related to the contaminated HB (C) and the moderately contaminated ALM (MC) (Fig. 6B). Cop and Total LABs had a larger multivariate dispersion opposite with the less contaminated sites CA16, CA70, and these sites had a positive correlation with both axes. Total AHs, UCM, and stig were correlated negatively with CAP1 and high positively with CAP2 according to the position of the most contaminated site MA (C).  $\Sigma$ PAHsc and PAHs 4-6 were positively correlated with CAP1, but  $\Sigma$ PAHsc was negatively correlated with CAP2 which marked differences in dispersion between sites CA16 and CA70, that are less contaminated than the other sites.





**Figure 6.** Canonical analysis of principal coordinates (CAP) ordination resulted from resemblance Jaccard matrix of athecate hydroids on coral reefs. Multiple correlation regression vectors: hydroid original species (A), organic molecular contaminants (B), Cont-sterol: factor level of contamination by fecal sterols, C-contaminated, CM-moderately contaminated and NC-no contaminated (groups of treatments).

### 3.4. Epiphytic athecate hydroid richness and frequency distribution in Vila Clara Seagrass meadows.

In the seagrass meadows seven species composed the hydroid fauna, classified in six genera and four families: Bouganvillidae, Oceaniidae, Corynidae and Eudendriidae. The last one was the best represented with three species (Table 6). The most frequent species were *Myrionema amboinense* with 26.1 % of frequency and *Eudendrium moulouyensis* con 17,9 %. The less frequent was *Eudendrium* sp. 1, *Coryne* sp1 and *Pachycordyle napolitana*, each one with the minimum value, 1.19 %.

Sites with high athecate richness were CAI\_2, CAI\_4, CAI\_5, and CAI\_7, with three species each. CAI\_1 and CAI\_12 had only one species each. *E. moulouyensis* appeared in all sites, being the only species found in the site's CAI\_1 and CAI\_12 with a minimum frequency of occurrence (8.33 %). Three species appeared in CAI\_2, CAI\_4, CAI\_5, and CAI\_7: the most abundant in CAI\_2 was *M. amboinense* (91.67 %) followed by *E. moulouyensis* (66.67 %) and ??? the less abundant (??). This same pattern was in CAI\_7 and CAI\_4, respectively 50 % and 33.3 % of frequency. In CAI\_5 the same three species had the minimum percentage of frequency.

**Table 6. Species richness (S) by site, absolute frequency (FA) and percentage of frequency of occurrence (%FO) of each species by site and by habitat in seagrass meadows, Villa Clara. CAI= Calbarien.**

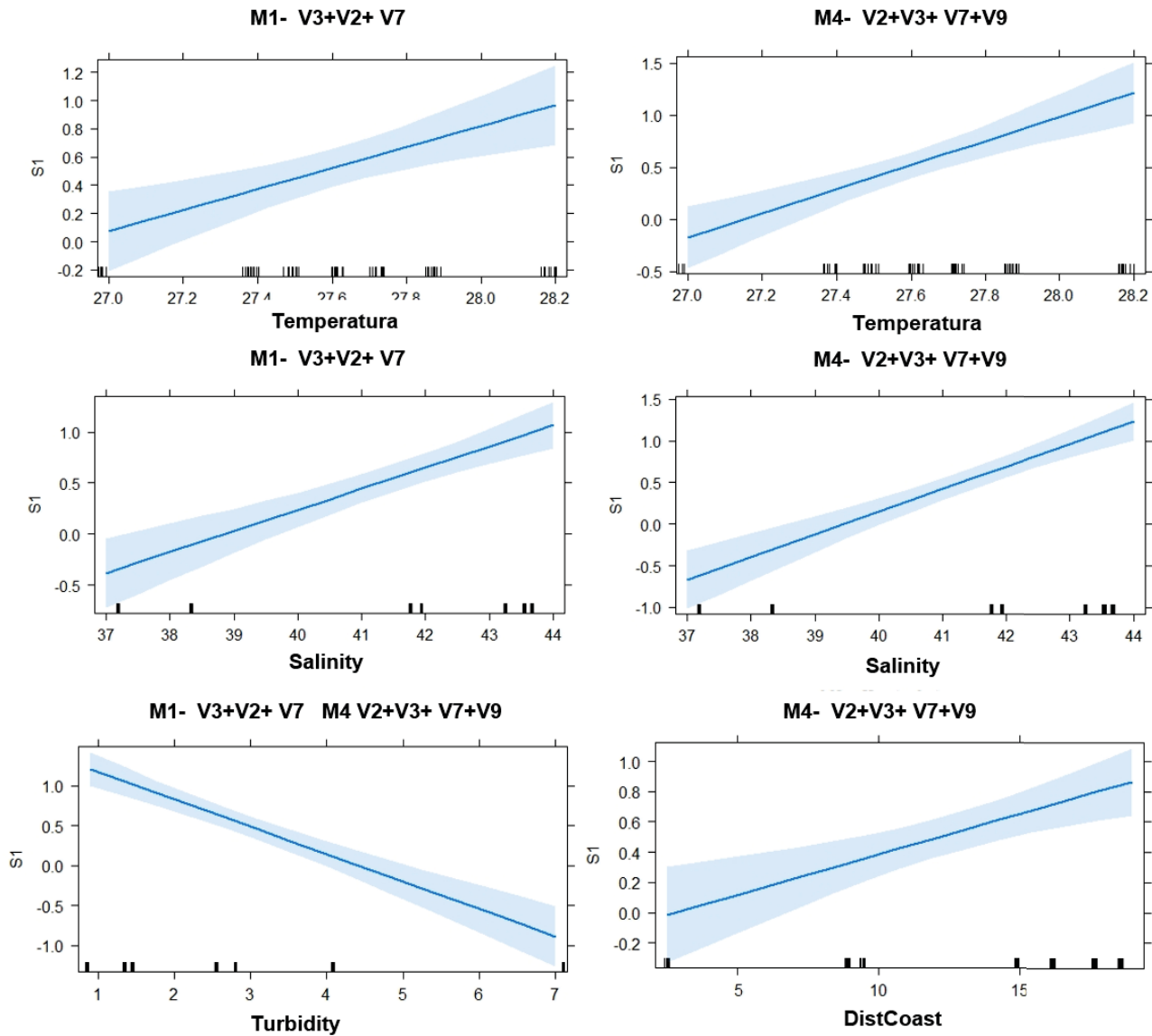
Taxon	Sites													
	CAI_1		CAI_2		CAI_3		CAI_4		CAI_5		CAI_7		CAI_12	
	FA	%FO	FA	%FO	FA	%FO	FA	%FO	FA	%FO	FA	%FO	FA	%FO
<b>Suborder Capitata Kühn, 1913</b>														
Family Corynidae Johnston, 1836														
<i>Coryne</i> sp 1	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	1,00	8,33	0,00	0,00	0,00	1,19
<b>Suborder Filifera Kühn, 1913</b>														
Family Eudendriidae L. Agassiz, 1862														
<i>Eudendrium moulouyensis</i> Marques, Peña Cantero and Vervoort, 2000	1,00	8,33	8,00	66,67	1,00	8,33	2,00	16,67	1,00	8,33	1,00	8,33	1,00	17,86
<i>Eudendrium</i> sp.1	0,00	0,00	0,00	0,00	1,00	8,33	0,00	0,00	0,00	0,00	0,00	0,00	0,00	1,19
<i>Myrionema amboinense</i> Pictet, 1893	0,00	0,00	11,00	91,67	0,00	0,00	4,00	33,33	1,00	8,33	6,00	50,00	0,00	26,19
Family Oceanidae Eschscholtz, 1829														
<i>Rhizogeton conicum</i> Schuchert, 1996	0,00	0,00	0,00	0,00	0,00	0,00	2,00	16,67	0,00	0,00	0,00	0,00	0,00	2,38
Family Bougainvilliidae Lütken, 1850														
<i>Millardiana longitentaculata</i> Wedler & Larson, 1986	0,00	0,00	1,00	8,33	0,00	0,00	0,00	0,00	0,00	0,00	1,00	8,33	0,00	2,38
<i>Pachycordyle napolitana</i> Weismann, 1883	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	1,00	8,33	0,00	1,19
<b>FA</b>	1,00		20,00		2,00		8,00		3,00		9,00		1,00	44,00
<b>S</b>	1,00		3,00		2,00		3,00		3,00		3,00		1,00	7,00



### *3.5. Relationship between hydroid richness and water quality variables*

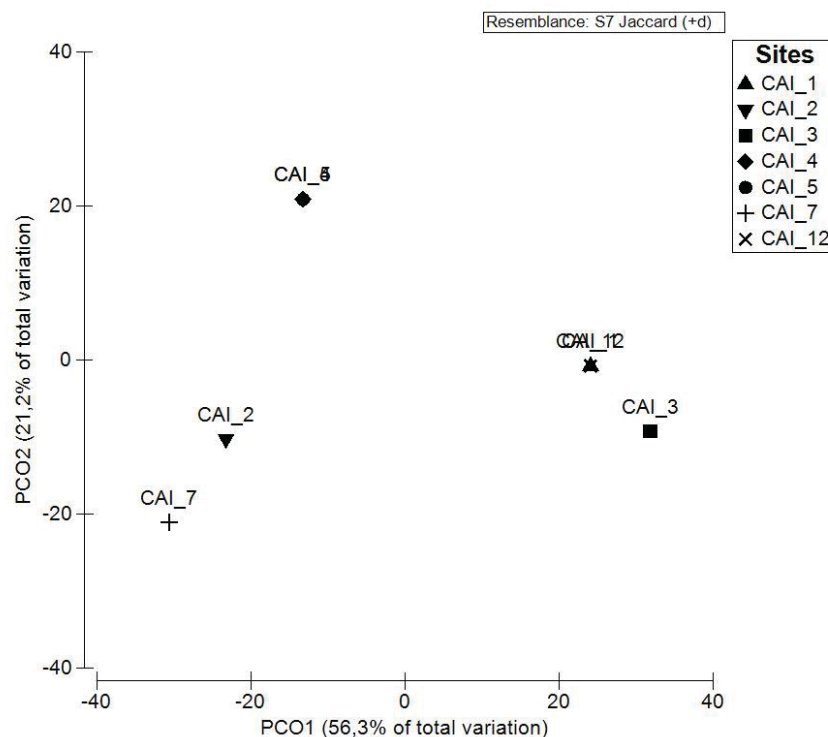
The GLMs resulted 266 models based on the effect of water quality predictors on species richness as response variable of hydroid athecate assemblage (see Table S2). Five models had the best fits for richness (S) and were also selected by delta AIC value (Table 7). The most explanatory model was M1 combining the variables salinity, temperature, and turbidity. Salinity and temperature were significant (p-value = 0.012 and p-value = 0.035, respectively) for the species richness response variable. The PH combined with salinity was significant (p-value = 0.010) in the M3 model. Between all predictor variables, the temperature showed the highest relative importance for predicting species richness of athecate hydroids in the seagrass meadows S (RI = 0.73), followed by salinity (RI = 0.73). Athebate richness increased with the increasing values of temperature, distance from the coast and salinity (Fig. 7). Despite not being significant, turbidity appeared in four of the five most explanatory models (M1, M2, M4, and M5) being its increment related to a decreasing richness of the athecates.





**Figure 7.** Effect of water quality variables in the most explanatory models on species richness (S) of athecate hydroids. M= Model, V2=temperature, V3= salinity, V7= turbidity, V9= distance from de coast.

As a result of PCO analysis, the first two principal components accounted for about 77.5 % of the total variation of species composition (Fig. 8). The unconstrained ordination of data variation was 56.3 % explained by PCO1 and 21.2 % by PCO2 axes. There was a greater multivariate dispersion of hydroid assemblages between CAI\_2 and CAI\_7 from the other sites related to PCO1. CAI\_5 and CAI\_4, the sites more distant from the main island coast, had a large separation from the other sites and had together the mainly correlation with PCO2. There is no difference in dispersion between CAI\_1 and CAI\_12 which are near to the higher impacted main island (Fig. 8).



**Figure 8.** Principal coordinates analysis ordination (PCO) based on presence-absence data and Jaccard resembles matrix of species athecate hydroids in seagrass meadows, Villa Clara. The codes for the sampled sites are presented in Table 1.

In the PERMANCOVA analysis, the covariates that better explained the hydroid assemblages were Temperature (6.7 %), Salinity (12.1 %), and DO (9.5 %), the last one with

the highest ECV. Turbidity explained 11.8 % of the community variation despite being near the borderline of the significant effect (p-value=0,08) (Table 8).

**Table 8.** Multivariate analysis of variance PERMANCOVA to compare sites with the covariates (water quality variables). \*indicate statistical significance at the 95 % level ( $p < 0.05$ ). Estimate of component of variation (ECV).

**Table 8 PERMANCOVA results:**

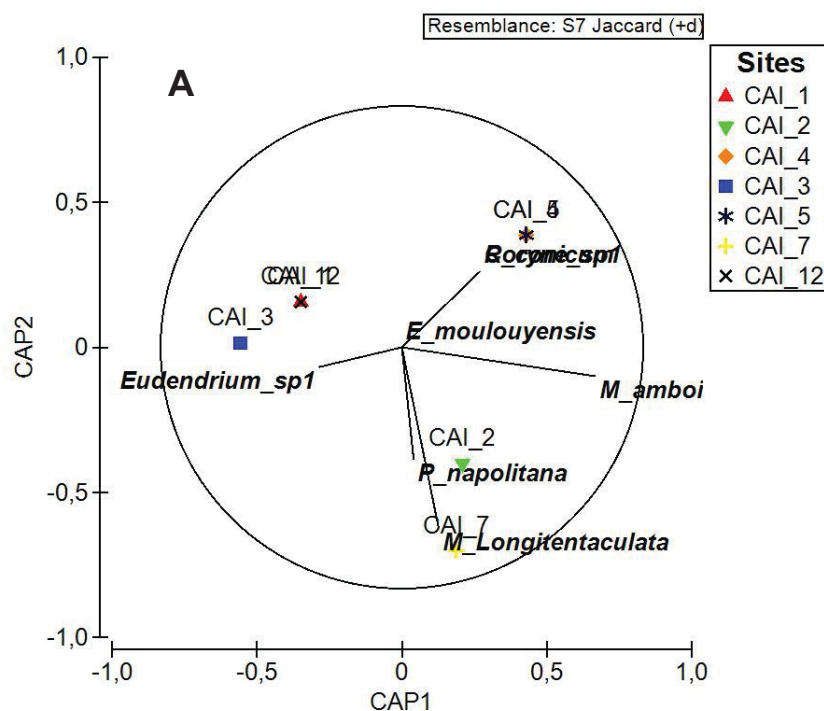
Covariates	df	SS	MS	Pseudo-F	P(perm)	perms	ECV (%)
Temperature	1	2636,6	2636,6	4,2528	0,0101*	9948	6.7
Salinity	1	4239,1	4239,1	6,8375	0,0005***	9947	12.1
PH	1	1277,8	1277,8	2,061	0,1163	9962	2.6
DO	1	19616	19616	31,639	0,0001***	9956	92.5
SO	1	424,56	424,56	0,6848	0,5439	9952	25.9
Turbidity	1	1414,6	1414,6	2,2817	0,0838	9971	11.8
COD	0	0		No test			
Distcoast	0	0		No test			
Depth	0	0		No test			
Site	0	0		No test			
Total	83	77347	619,98				

Legend: depth (m), salinity (ups), turbidity (FTU), temperature (°C), pH, oxygen saturation (%) (SO), dissolved oxygen (DO) (mg.L-1), chemical oxygen demand (COD) (mg.L-1), and coast distance (Distcoast) (km)

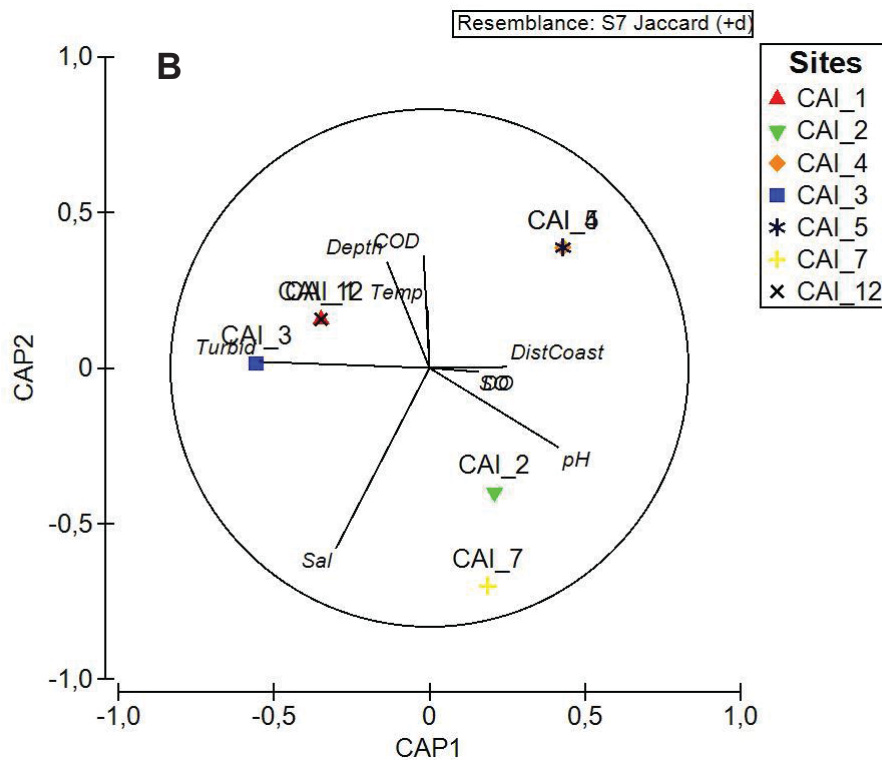
Canonical analysis of principal coordinates (CAP) explained a multivariate constraint spatial ordering (98 %) for the epiphytic athecate hydroids in seagrass meadows (Fig.9A). The hydroid *Myrionema amboinense* had the highest correlation with the CAP 1 axis (0.80), marking the separation between sites CAI\_4, CAI\_5, CAI\_2 and CAI\_7 from sites CAI\_3, CAI\_1 and CAI\_12. *Pachycordyle napolitana* and *Millardiana longitentaculata* were also positively correlated with CAP1 and negatively with CAP2 related with the spatial position of CAI\_2 and CAI\_7, the unique sites where these species appeared. *Eudendrium* sp.1 was negatively correlated with both axes and related with the position of CAI\_3. The negative correlation with CAP1 is also observed for CAI\_1 and CAI\_12. *Eudendrium moulouyensis* contributed with the similarity of these two sites despite its presence in all sites. The canonical correlation with CAP2 showed a variation in dispersion of the group CAI\_4 and CAI\_5 correlated positively with both

axes. These sites were separated from the others because *Rhizogeton conicum* (CAI\_4) and *Coryne* sp.1(CAI\_5) were exclusive of them.

Based on the water quality and the distance of the main island, the CAP showed a large variation with respect to the canonical correlation axes for environmental covariates between sites (Fig.9B). The correlation with CAP1 showed a clear separation of sites with better water quality (CAI\_4, CAI\_5, CAI\_2, and CAI\_7) from those with poorer conditions (CAI\_3, CAI\_1, and CAI\_12), marked by the variables Turbidity (Turbid) and distance from the coast (DistCoast) with contrary correlation with the canonical axes. Besides DistCoast, DO and SO differentiated the position of the sites with better environmental conditions. Turbidity was the variable with the highest correlation with canonical axis CAP1 and Salinity with CAP2, both negatively correlated. PH was also negatively correlated with CAP2 in relation with sites CAI\_2 and CAI\_7. Depth and COD had a high positive correlation with CAP2, related with the dispersion of sites CAI\_1 and CAI\_12 which had the highest influence of contamination from the main island.







**Figure 9.** Canonical analysis of principal coordinates (CAP) analysis of athecate hydroids on seagrass using Jaccard Index with quadrants, with different numbers of samples. Multiple correlation regression vectors: hydroid original species (A), water quality variables (B). **Legend:** Sites and variables codes see table 1 and table 8.

#### 4. Discussion

In this study a total of eighteen species of athecate hydroids were found, twelve in coral reefs and seven in seagrass meadows. The number of athecate hydroids in Cuban waters is similar to that reported for other places around the world by Galea (2008, 2013) for rocky shores, coral reefs, and seagrass in the islands of Guadeloupe and Martinique in the Caribbean and by González-Duarte et al. (2013) in hard bottom assemblages in the Atlantic-Mediterranean and the African–European axes. Otherwise, the number of athecate hydroids of this study is low when compared to the number of thecate hydroids reported for the same areas (Castellanos-Iglesias et al., 2018) in coral reefs of Havana and in seagrass meadows, Villa Clara (Castellanos-Iglesias 2017), but this is in absolutely agreement with the number of

species described for these two groups in the world, that maybe explained by the presence of the theca, a protective structure where the hydranth can withdraw (Gili and Hughes, 1995).

Our null hypothesis of no differences in species richness of athecate hydroids between sites was reject, either for the coral reefs or seagrass meadows, both habitats influenced by anthropogenic impacts. Similar results of variation in the structure of benthic communities influenced by contamination were found for thecate hydroids in the same study areas by Castellanos-Iglesias et al. (2018) in Havana and by Castellanos-Iglesias (2017) in Villa Clara. Changes in the structure of coral communities associated with the anthropogenic impacts have been detected along the northwestern coast of Cuba (Gonzalez, 2010). Hernández-Muñoz et al. (2008) also found changes of the influence of urban residuals on the octocoral communities in Havana between the years 2002 to 2005. Gonzalez-Diaz et al. (2018) found differences in benthic communities of corals, sponges, and gorgonians associated to urbanized areas in Havana. Rey-Villiers et al. (2020a) also detected changes in octocoral communities related to the deterioration of water quality and fecal organic pollution, in the northwestern region of Cuba. In other regions of the world as in the Babitonga Bay, Brasil, Cabral (2013) detected hydroid species (*Garveia franciscana*, *Ectopleura* spp., and *Obelia dichotoma* as indicators of environmental conditions.

On Havana's coral reef we found the highest athecate hydroid species richness in the contaminated site HB, so, we also reject our hypothesis that diversity of athecate hydroid diminishes in sites with high stress of contamination. This result is opposite to that for thecate hydroids assemblages, which have the second minor value of richness in the same site (Castellanos-Iglesias et al., 2018). Similar to our results were those obtained for other marine groups such as corals (Alcolado et al., 2012), with the highest presence of species resistant to organic contamination, sedimentation and abrasion in the most polluted site in Havana, and also for sponges (Alcolado, 2007; Busutil and Alcolado, 2012), that evaluated the potential for environmental bioindication in pollution-tolerant sponge species.

The diversity of athecate hydroids decreased in the not contaminated site SA, the farthest site from the Port of Havana which had the lowest values of faecal contamination by coprostanol. Similar results were also found along the southern Iberian Peninsula (Megina et al., 2013) where similar richness values were observed in communities of hydroids

assemblages in harbors and in natural habitats. The moderately contaminated site CA16 had the second highest value of species richness of athecate hydroids, similar result was found for thecate hydroids with the highest richness in this same site (Castellanos-Iglesias et al., 2018). It was observed in Havana coral reefs that athecate species richness increased with the increment of cholesterol. Cholesterol and stigmasterol signalize the presence of organic matter of natural origin as from zooplankton and phytoplankton communities (Volkman, 2005). Cholesterol and stigmasterol values were higher in moderately contaminated and uncontaminated sites (Martins et al., 2018). Similar results happened with the thecate assemblages in the same area (Castellanos-Iglesias et al., 2018).

The site HB with highest species richness in this study, has the highest concentrations of coprostanol and LABs (linear alkylbenzenes). Coprostanol is an indicator of sewage input, and its degradation under anoxic conditions in sediments occurs very slowly (Bachtiar et al., 2004). Sewage discharges tended to benefit suspension feeders (Cabral-Oliveira et al. 2014), such as hydroids. According to Boero (1984) some species of hydroids are resistant to poor water quality conditions and pollutants can stimulate colonial growth. Considering that the frequency of occurrence of species at all sites was low, it is likely that the combination of these compounds caused a decrease in abundance but not in species richness at this site, as found by Cifuentes et al. (2007) in Herradura Bay in northern-central Chile for macrobenthic fouling organisms.

LABs as compounds present in commercial detergents, are frequently discharged via sewage and conserved in sediments for 10 to 20 years (Eganhou et al., 1983, Martins et al., 2014). They were selected in two of the best fit models of the GLM analysis for the coral reefs, as significative variable to explain richness of athecate hydroids, combined with coprostanol in one of the models, and with cholesterol, stigmasterol and Total AH in the other. The most contaminated sites HB and MA had the highest concentrations of LABs. Lewis (1991, 1992) made a list of the effects of chronic toxicities of surfactants to aquatic invertebrates reported in the literature related to the survival, reproduction, growth, egg development, among others. Also, Marin et al. (1991) detected in laboratory that concentrations of a surfactant in water affected the survival and growth rates of benthic filter feeder's sea-squirts.

Anthropogenic pollution is associated with a decrease in species richness in marine habitats (Johnston and Roberts, 2009). Sites nearby Havana Bay are affected by high levels of PAHs and of UCM, causing toxic effects to aquatic organisms (Martins et al., 2018). Urban runoff and sewage effluents are among the main sources of PAH in the coastal marine zone, some of these compounds may be carcinogenic or mutagenic (Latimer and Zheng, 2003). It has been studied that this contaminant has negative effect in other benthic organisms as the Pacific oyster, *Crassostrea gigas* (Gagnaire et al., 2006). PAHs\_4\_6 rings were selected in the best fit model as a predictor variable for athecate richness. HB and MA had the highest concentration of this compound. Probably the highest richness found in HB, the most contaminated site, is related to the presence of species tolerant to these contaminants. Millward et al. (2004) studied the effects of pollutant mixtures on a saltmarsh benthic invertebrate community and found that community responses to pollutant mixtures are a function of the species composition and/or the relative concentrations of pollutants in the mixtures.

According to our results the combination of the high concentrations of coprostanol and UCM could be one of the causes of the low diversity of athecate hydroids in MA. This site, with less species richness of athecate hydroids has the second highest concentration of coprostanol and the highest concentration of UCM (Martins et al. 2018). Castellanos-Iglesias et al. (2018) also found that fecal contamination mainly coprostanol affected the thecate hydroid assemblages' distribution in the most contaminated sites. UCM are oil-derived pollutants persistent in sediments that can cause changes in larval mating or settlement and when in high concentrations cause toxic effects such as narcotic stress in marine life (Brownawell et al., 2007). Anthropogenic compounds can affect the defense capacity of marine animals and increase their susceptibility to high concentrations of them (Meador, 1995).

Our hypothesis related to seagrass meadows, which predicts a lower diversity of athecate hydroids closer to the main island, and greater diversity in sites far from the main island, marked by a gradient of water quality, was not rejected. The richness of athecate hydroids was mainly related to the combination of salinity, temperature, turbidity, and distance from the coast. According to Calder (1992) water temperatures in the western North Atlantic become more stable with increasing distance from the coast. He found that the number of tropical hydroid

species increased with depth related to the differences in temperature between the inshore and offshore.

Salinity was significant for the models M1 and M4 from the GLM and the graphics (Fig.7) showed that increases in salinity are related to an increase in the species richness of athecate hydroids. Calder (1976) found that salinity is the main factor that determines the hydroids assemblages in south Carolina Estuaries and that hydroids tolerant to variations in salinity are also tolerant of some types of contamination stress. In estuaries of the state of Sergipe, Brazil, Castro (2020) also found that richness and abundance of hydroids varied proportionally with the variations of salinity. Changes in salinity can also affect larval growth and consequently the recruitment success in benthic macroinvertebrates as was studied in a South Carolina (USA) estuary (Richmond and Wooding, 1996).

Dissolved oxygen (DO) had the highest component of variation to explain the structure of hydroids assemblages in the seagrasses, followed by salinity and temperature. Hydroids are common and abundant in environments with oxygen concentrations greater than 5 mg L<sup>-1</sup> (Gili and Hughes, 1995). The lowest DO value was found in the CAI\_1 site (4.05 mg L<sup>-1</sup>), one of the two sites with the lowest species richness. The other sites values were closer to or greater than 5 mg L<sup>-1</sup>. DO values are partially related to the photosynthesis of the phanerogams influencing water quality. Turbidity is one of the factors that diminish the availability of light on the seagrasses (Barsanti et al., 2007), causing negative impacts on this marine environment (Martínez-Daranas and Suarez, 2018).

Based on the water quality and the impact of pollution from the main island, the variables turbidity (Turbid) and distance from the coast (DistCoast) marked the differences between the sites with higher (CAI\_2, CAI\_4, CAI\_5, CAI\_7) and lower (CAI\_1 and CAI\_12) diversity of athecate hydroids. In the farther sites from the coast, the salinity and turbidity are lower, due to increased exchanges with the ocean generating better water quality condition, while in the most internal areas of the bays, the organic matter is retained leading to an increase in turbidity (Betanzos-Vega et al., 2011; Martinez-Daranas et al., data not published).

*Myrionema amboinense* was the most abundant species that best contributed to the separation between the sites (CAI\_2, CAI\_4, CAI\_5, CAI\_7) and (CAI\_1 and CAI\_12) in

seagrass meadows, as it is present only in the first group (CAI\_2, CAI\_4, CAI\_5, CAI\_7), which had better water quality conditions (less turbidity, high light intensity and adequate concentrations of dissolved oxygen). Probably its symbiosis with zooxanthellae favors the abundance in most clear waters. Maybe this species also presents sensitivity to organic organic matter from the impacted main island since its sensitivity to oil pollution has been reported by Michel and Fitt (1984) and Michel (1985) (as *Myrionema hargitti*).

In general, the distribution and diversity of the athecate hydroid assemblages here studied were sensitive to the variation of chemical molecular markers that indicate organic contamination by sewage, oil, and detergents in the Havana coral reefs. Athecates were also sensible to the variations of physical factors of water quality conditions such as turbidity, salinity, temperature, and dissolved oxygen in the seagrass meadows. Human impact assessed by distance from the coast was a good predictor of species richness of athecate hydroids supporting the importance of this group as environmental indicators.

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### Supplementary material

**Table S1. Environmental data of hydrochemistry in the sampling sites of Havana coral reefs where hydroids were collected. Concentrations (in  $\mu\text{g g}^{-1}$ ) of coprostanol (cop), Cholesterol (chol\_e), stigmaterol (stig), total aliphatic hydrocarbons (Total AHs), unresolved complex mixture (UCM), Polycyclic aromatic hydrocarbons (PAHs), and Total Linear Alkylbenzene (Total LABs). lat= latitude, long= longitude.**

sites	cop	chol_e	stig	Total AHs	UCM	$\Sigma\text{PAHsc}$	PAHs_4_6	Total LABs
HB	1,37	0,97	0,38	86,5	77,6	2133	1840	22,7
MA	0,93	0,77	0,4	135,7	124,8	1707	1417	13,4
ALM	0,53	4,24	1,43	71,5	61,6	159,8	131,7	6,67
CA16	0,33	5,58	1,68	35,9	30,1	197	159,4	0,76
CA70	0,05	0,31	0,28	13,8	11,9	85,2	76	0
EM	0,21	0,56	0,36	20	17,1	151	11,2	0
SA	0,02	1,64	0,85	1,51	0	1,96	0	0

**Table S2. Environmental data of hydrochemistry in the sampling sites from seagrass meadows where hydroids were collected. Temp= temperature, sal= salinity, DO= dissolved oxygen, SatO2= oxygen saturation, Turb= turbidity, COD= chemical oxygen demand, DistCoast= coastal distance.**

Bays	Sites	Depth m	Temp °C	Sal (ups)	DO (mg/l)	SatO2 (%)	pH	Turb. FTU	COD (mg.L <sup>-1</sup> )	DistCoast km
SRB	CAI_1	3.2	27.52	45.2	3.49	58.4	8.14	6.42	0.72	2,5
SRB	CAI_2	2	27.79	43.33	4.98	80	8.19	1.35	1.26	9,4
SRB	CAI_3	2.5	28.17	43.67	4.89	86.4	7.96	7.1	3.79	8,9
SRB	CAI_4	2.6	27.72	38.34	5	80.8	8.3	1.45	1.44	16,2
SRB	CAI_5	2.8	27.57	37.34	6.3	98	8.01	0.86	1.80	17,7
SRB	CAI_7	2.5	26.98	43.64	5.9	90.6	8.28	2.81	0.72	18,6
BVB	CAI_12	2.7	27.38	41.94	5.69	88	8.03	4.08	1.26	14,9

## CONCLUSÕES GERAIS

Esta tese teve como objetivo ampliar o levantamento e atualizar os registros da fauna de hidroides atecados de Cuba e avaliar a diversidade desses organismos em relação à variação no estresse de contaminação e às condições de qualidade da água, nas regiões norte e centro de Cuba em habitat de recifes e gramas marinhas.

Do estudo da taxonomia dos hidroides atecados foram reportadas 20 espécies das quais 11 foram novas ocorrências no país. As espécies *Eudendrium molouyensis* e *Rhizogeton conicum* foram novos reportes para o Caribe. O recife de coral foi o habitat mais especioso do que as gramas marinhas. Como resultado deste estudo, a lista de hidroides atecados de Cuba aumentou de 24 para 35 espécies, para 5 dessas espécies, estruturas reprodutivas são descritas pela primeira vez para o país.

A partir da análise da relação das comunidades de hidroides atecados com os níveis de contaminação e condições de qualidade da água, foi possível verificar que a riqueza de espécies variou em resposta às variações ambientais nos locais de estudo.

Como recomendação, propõe-se que futuras pesquisas incluam coleções na região leste do país para aumentar o conhecimento da distribuição dos hidroides em Cuba.